



Surveys of Amphibians, Abnormalities, Pathogens, Triazines, Breeding-Site Characteristics, and Reptiles in Five Areas Managed by the National Park Service and the U.S. Fish and Wildlife Service in the Upper Midwest, 2002-2007

Natural Resource Technical Report NPS/GLKN/NRTR—2009/179



ON THE COVER

Northern spring peeper found during surveys along the St Croix National Scenic Riverway.
Photograph by Mark Roth, U.S. Geological Survey.



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Executive Summary

Amphibian and reptile populations have been declining in many locations around the world. We are concerned not only because these populations have intrinsic ecological value, but also because the sensitivity of these populations to environmental change underlies their declines and further environmental changes could cause further declines. We also are concerned because the environmental changes responsible for these declines may have broader effects on ecosystem function, overall biodiversity, ecological services, and human health. Thus, understanding the statuses of amphibian and reptile populations in relation to changing environmental conditions is important for many reasons.

We and our colleagues from the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin, and Minnesota Water Science Center in Mounds View, Minnesota, conducted the work described in this report. We worked on lands managed by the U.S. Department of the Interior (DOI) in support of the goals of the USGS Amphibian Research and Monitoring Initiative (ARMI) and the U.S. National Park Service (NPS) Inventory and Monitoring Program (IMP), especially as they applied to the Midwest Region of ARMI (ARMI-MR) and NPS Great Lakes Inventory and Monitoring Network (GLKN). These goals were to assess the statuses of amphibian populations on DOI lands relative to key environmental factors in ARMI Midwest Region and to inventory Voyageurs National Park (VOYA), the St. Croix National Scenic Riverway (SACN), and the Mississippi National River and Recreation Area (MISS) for the presence of amphibian and reptile species. Thus, we designed surveys to meet the goals of both programs.

We also conducted surveys in two areas managed by the U.S. Fish and Wildlife Service (FWS): the Upper Mississippi River National Wildlife and Fish Refuge (UMR) from 2002-2005 and the Neal Smith National Wildlife Refuge (NS) during 2004 and 2005. We surveyed these refuges to provide ARMI-MR and FWS with fundamental information on amphibian diversity relative to environmental conditions, with a goal to better manage natural resources on FWS refuges. We have included data from our work in the UMR and the NS in this report to provide a regional context for the data from NPS lands.

Our principal objectives throughout these studies were to 1) conduct visual-encounter and call surveys for amphibians at potential breeding sites in VOYA, the SACN, the UMR, and the NS, and targeted searches for amphibians in the MISS, 2) conduct visual-encounter surveys for reptiles, especially turtles, in the vicinity of amphibian breeding sites in VOYA and the SACN, and to a lesser extent, the MISS, 3) analyze amphibian survey data across management units and years using information-theoretic models to estimate occupancy and the probability of detection in relation to site-specific and sampling-specific characteristics, 4) sample water from amphibian breeding sites in VOYA, the SACN, the UMR, and the NS and analyze for the presence of triazine herbicides, 5) measure gross physical abnormalities among amphibian metamorphs at breeding sites in VOYA, the SACN, the UMR, and the NS, and 6) sample amphibian populations in VOYA, the SACN, and the UMR for the presence of pathogens, parasites, and symptoms of disease.

We surveyed in VOYA and the SACN from 2002-2007, in MISS during 2003 and 2004, in the UMR from 2002-2005, and in the NS from 2003-2005.

A summary of our principal results is as follows:

- We observed most of the amphibian and reptile species we expected to find in each management unit given known historical distributions, species we targeted, resource limitations, and the methods we employed. We found 84%, 75%, 72%, 71%, and 31% of the amphibian species potentially present at SACN, NS, UMR, VOYA, and MISS (where we targeted fewer species and devoted less resources). Similarly, we observed 43% of the possible snakes and 67% of the possible lizards in the SACN and 75% of the possible snakes in VOYA. No lizards were likely in VOYA, and we did not observe any.
- Estimates of occupancy and probabilities of detection varied widely for amphibian species sampled during the day across VOYA, the SACN, the UMR, and the NS. Occupancy estimates ranged from 0.29 (western chorus frog) to 0.79 (green frog) with standard errors for occupancy from 0.05 (northern green frog) to 0.9 (eastern American toad). Ranges for the probabilities of detection were from 0-0.34 (eastern American toad) to 0.18-1.0 (green frog) with standard error ranges for detection probabilities from 0-0.13 (green frog) to 0-157.8 (northern spring peeper). Sampling-specific covariates that were influential in the best-fitted occupancy models were time, observer, and date. Similarly, site-specific covariates were management unit and habitat type.
- Of the 523 water samples we collected at amphibian breeding sites across management units and analyzed for the presence of triazines, 30.8% were below the detection limit of 0.050 ppb, mostly in the SACN and VOYA. Fifty-five percent of the values above the detection limit also were above 0.1 ppb, a threshold shown to cause deformed gonads in frogs in the laboratory; most of these were samples from the NS and UMR. The relative triazine concentrations across management units followed their proximity to extensive areas of row crops, where triazines are applied in large quantities. Triazine concentrations at breeding sites in the floodplain of the Mississippi River in the UMR fluctuated in association with the hydrograph of the Mississippi River.
- We observed gross physical abnormalities in less than 5% of the metamorphs sampled in VOYA, the SACN, the UMR, and the NS. Percentage of abnormal metamorphs across all species for VOYA, the UMR, and the SACN from 2002-2005 ranged from 0.5% to 4.5%, and means for each of these management units across years were 1.5, 2.4, and 1.4%, respectively. These rates did not appear to be cause for alarm.
- We observed the potentially deadly chytrid fungus, *Batrachochytrium dendrobatidis*, on green frogs and mink frogs from practically all sites where we sampled them in VOYA (88%) and the SACN (100%) during 2006, but at fewer sites on northern leopard frogs in the UMR (25%). The pattern was similar for these species in 2007, except we also sampled green frogs in the UMR. We observed *B. dendrobatidis* on green frogs and mink frogs at most sites where we sampled them in VOYA (71%) and the SACN (67%), and the majority of sites where we sampled green frogs in the UMR (80%). In contrast, we observed *B. dendrobatidis* on

northern leopard frogs at only 13% of the sites where we sampled them in VOYA, the SACN, and the UMR. These results suggest that amphibians are exposed to this pathogen in a substantial proportion of the wetlands in VOYA, the SACN, and the UMR, but the fungus is not present equally on all species. We also observed chytridiomycosis, the disease this pathogen causes, in a small number of mink and green frogs from three sites in VOYA.

Most individual, apparently healthy amphibians we submitted for health screening were afflicted with parasites of one sort or another, ranging from flukes to trematodes. *Ribeiroia ondatrae*, a parasitic trematode we observed, is considered a primary inducer of gross physical abnormalities in amphibians.

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Introduction

Coordination of Research Efforts

The National Park Service (NPS) Inventory and Monitoring Program (IMP) is intended to establish baseline information on natural resources in parks and long-term monitoring of those resources in relation to environmental conditions (NPS 2007). NPS began implementing the IMP in the Great Lakes Network (GLKN; GLKN 2007) in 2001.

One of the GLKN's initial goals was to confirm the presence or absence of at least 90% of the possible resident species of amphibians and reptiles in GLKN parks. We were contracted by the GLKN in 2002 to conduct the surveys necessary to address this goal. From 2002-2004, we leveraged USGS funds (>50%) from the Amphibian Research and Monitoring Initiative's Midwest Region (ARMI-MR) with NPS GLKN funds to survey resident amphibians and reptiles (primarily turtles) in Voyageurs National Park (VOYA), the St. Croix National Scenic Riverway (SACN), and the Mississippi National River and Recreation Area (MISS) while also meeting ARMI's goals.

This report includes the results of those surveys. However, our work in the GLKN was also a more comprehensive overview of the statuses of amphibian populations in VOYA and SACN relative to environmental conditions and was linked to similar work we did on other DOI lands in the ARMI-MR. Thus, we expanded this report to include a wider set of data from 2002-2007 that includes information for the Upper Mississippi River National Wildlife and Fish Refuge (UMR) bordering Illinois, Iowa, Minnesota, and Wisconsin, and the Neal Smith National Wildlife Refuge (NS) in Iowa. These additional data resulted from our larger efforts in the ARMI-MR, supported completely by USGS-ARMI funds, and were beyond the scope of the original agreement with the NPS. This additional information may prove useful to resource managers in the NPS and the U.S. Fish and Wildlife Service (FWS) by establishing a broader context in which to consider statuses of amphibian populations and environmental conditions in their management units.

Why Do We Care About Amphibians and Reptiles?

This report pertains primarily to issues regarding the current and future statuses of amphibian and reptile populations in specific parks and refuges in the midwestern United States. These issues are important for at least two reasons:

1. Amphibians and reptiles have declined in many areas around the world (e.g., Gibbons et al. 2000; Stuart et al. 2004). They have declined because they are sensitive to changes in environmental conditions that often are summarized as global change (see next section). While these declines are part of a larger decline in biodiversity, amphibians and possibly reptiles have declined more dramatically than most other taxa. For example, Stuart et al. (2004) reported the following for amphibians:
 - 9 species are known to have gone extinct since 1980,
 - 113 species likely are extinct,
 - 1,856 of 5,743 species (32%) currently are threatened with extinction (compared to 12% of birds and 23% of mammals), and
 - 43% of species are considered to be in decline.

Their report was part of the Global Amphibian Assessment based upon input from 500 scientists from 60 countries. A similar global assessment is underway for reptiles. Many believe reptiles are faring at least as poorly as amphibians (Gibbons et al. 2000), but we simply do not know as much about reptile populations. Amphibians and reptiles are key components of food webs, productivity, and ecosystem services in ecological systems (Johnson 2006). Given this importance, understanding the statuses of amphibian and reptile populations in the face of global change is critical to managing and conserving the nation's biological resources.

2. The changes in ecological systems causing amphibian and reptile declines also are affecting other organisms (Halliday 2005). In addition to a worldwide loss of biodiversity, humans are among the organisms that can be affected in a variety of ways, including via direct effects on our health or indirect effects of degraded ecosystem services we require to live (Reaser and Blaustein 2005).

Thus, understanding the nature and implications of ecosystem changes causing effects on sensitive amphibian and reptile populations can help us understand potentially important effects on all organisms, including ourselves. Integrated and comprehensive research that provides useful information on amphibian and reptile populations in relation to global change is needed by the general public, scientists, landowners, resource managers, regulators, and policymakers.

Declines of Amphibian Populations

Declining amphibian populations around the world have concerned conservation biologists since the 1980s (Hayes and Jennings 1986; Barinaga 1990; Bradford 1991; Carey 1993; Fellers and Drost 1993; Kagarise Sherman and Morton 1993; Blaustein 1994; Drost and Fellers 1996; Lannoo 1998; Lips 1998; Knapp and Matthews 2000; Reaser 2000; Alexander and Eischeid 2001; Carey et al. 2001; Young et al. 2001; Stuart et al. 2004; Lannoo 2005; Lips et al. 2006; Pounds et al. 2006; Davidson and Knapp 2007). Ultimately, these declines are due to changed environmental conditions and unique life-history traits that render amphibians sensitive to such changes (Halliday 2005). Because of their sensitivity, amphibians are recognized widely as potentially valuable bioindicators (Heyer and Murphy 2005; Reaser and Blaustein 2005) and their declines as indicative of widespread environmental changes (Blaustein and Dobson 2006; Pounds et al. 2006).

Environmental stressors that can cause declines include loss of habitat, disease, pollutants, climate change, ultraviolet radiation, and competition and predation by introduced species, among others (Bradford 1991; Griffiths and Beebee 1992; Blaustein et al. 1994a; Bradford et al. 1994a; Pounds and Crump 1994; Carey and Bryant 1995; Kupferberg 1997; Berger et al. 1998; Adams 2000; Carey 2000; Knapp and Matthews 2000; Alford et al. 2001; Bosch et al. 2001; Davidson et al. 2001; Kiesecker et al. 2001; Gillespie 2001; Pounds 2001; Blaustein and Kiesecker 2002; Hayes et al. 2003; Petranka et al. 2003; Stuart et al. 2004; Bosch and Martinez-Solano 2006; Pounds et al. 2006; Davidson and Knapp 2007). The relevance and intensity of environmental stressors vary in space and time and tolerances to those stressors vary with individuals, populations, and species (Griffiths and Beebee 1992; Alford et al. 2001; Blaustein and Kiesecker 2002; Halliday 2005; Crump 2005). Given inherent fluctuations in amphibian population dynamics, quantifying declines can take many years (Griffiths and Beebee 1992;

Pechmann and Wilbur 1994; Blaustein and Kiesecker 2002). Even when a decline has been described, demonstrating its relationship to specific stressors at requisite multiple scales can be daunting (Corn and Fogelman 1984; Griffiths and Beebee 1992; Davidson et al. 2001; Young et al. 2001; Blaustein and Kiesecker 2002). Scientific approaches to these challenges require accumulating complementary evidence in support or rejection of specific hypotheses pertaining to both the status of a population and detrimental impacts of specific stressors on that population. Given the complexity of these issues, the cumulative lines of evidence necessary to unequivocally link a decline to specific causes can prove elusive, especially for declines that occurred in the past.

An additional challenge to quantifying declines is that few long-term integrated population studies were conducted historically, or have been ongoing, to establish baseline data (Lannoo 1998; 2005). Such data are necessary to establish statuses and trends and to understand the environmental context of fluctuations in populations over time. This is true whether the goal of such research is to help conserve populations or to study amphibians as indicators of effects on other organisms. Given that we know relatively little about the statuses of most amphibian populations, we must at least initiate the process of documenting where species are present on the landscape and monitoring populations and relevant environmental conditions over time.

The U.S. Geological Survey's Amphibian Research and Monitoring Initiative (ARMI)

ARMI was created in 2000 to study statuses of amphibian populations and causes of declines across regions of the United States (Muths et al. 2005; ARMI 2007; ARMI-MR 2007; Figure 1). ARMI scientists have developed, tested, and implemented various methods to study amphibians relative to environmental conditions (ARMI 2007) and are working, along with partners, to collect data that are comparable across regions, meaningful in interpretation, and comprehensive in scope (Muths et al. 2005). Considerable effort has been invested in developing standardized sampling procedures, designing a national database, and ensuring that data collected will be comparable and available ultimately to those who need them in usable and useful formats.

ARMI in the Midwest Region

Approximately 89 species and subspecies of amphibians live in the Midwest Region of ARMI (Conant and Collins 1998; Lannoo 1998; Fischer et al. 1999; NatureServe 2004; KSNPC 2004; SRK 2004; Potts and Collins 2005; ARMI-MR 2007; University of Nebraska 2007). Because of the relatively little federal land in the ARMI-MR (Data Supplement-Figure 1), most of these amphibians do not live on federal lands, and even fewer live on the subset of federal lands managed by the Department of Interior (DOI) for the conservation of natural resources. Thus, most amphibian populations in the ARMI-MR face a different set of stressors than those living on DOI-managed lands. However, amphibian populations often straddle DOI boundaries, so any stressor effects on these populations in the non-DOI portion of the range could have significant implications for population dynamics as a whole.

Some amphibian populations are of known conservation concern in the ARMI-MR (ARMI-MR 2007), and many face some of the same threats as populations in other parts of the world



Figure 1. Regions of the USGS Amphibian Research and Monitoring Initiative in the conterminous United States. Red dots indicate USGS science centers.

(Lannoo et al. 1994; Lannoo 1998, 2005). However, recent dramatic declines have not been reported as widely from the ARMI-MR as from the western United States, for example (Corn and Fogleman 1984; Lannoo 1998; Bradford 1991; Carey 1993; Kagarise Sherman and Morton 1993; Bradford et al. 1994b; Drost and Fellers 1996; Lannoo 1998; Knapp and Matthews 2000; Reaser 2000; Carey et al. 2001; Davidson and Knapp 2007). This could reflect a combination of less dramatic recent declines, current stability among populations, a lack of appropriate data to describe declines adequately, or combinations of these factors and others. Many of the declines in the western states have been observed in mountainous regions where relatively long-term, large-scale studies have been conducted (Carey 1993; Kagarise Sherman and Morton 1993; Blaustein et al. 1994; Bradford et al. 1994b; Kiesecker and Blaustein 1995; Drost and Fellers 1996; Knapp and Matthews 2000). Few comparable studies have been reported thus far from the ARMI-MR (Lannoo 1998; Moriarity 1998).

We do not understand historical variation (Pechmann and Wilbur 1994) or amphibian population statuses (Lannoo 1998) well enough to describe current population levels or predict their trajectories for populations in the ARMI-MR. For example, populations of leopard frogs (*Rana pipiens* Complex) and Blanchard's cricket frogs (*Acris crepitans blanchardi*) have declined or gone extinct over the past several decades (e.g., Gibbs et al. 1971; Hine et al. 1981; Corn and Fogleman 1984; Lannoo et al. 1994; Broadman and Kilmurry 1998; Hay 1998; Lannoo 1998; Mierzwa 1998; Moriarity 1998). Unfortunately, most remaining populations have not been monitored rigorously enough to characterize trends in their distribution or abundance over time (e.g., Casper 1998; Moriarity 1998).

Surveys and monitoring have been ongoing or initiated recently in this region. For example, state agencies or other organizations in most states of the ARMI-MR, along with some federal

agencies and bureaus, such as the U.S. Forest Service, the FWS, and the NPS manage or conduct amphibian surveys (e.g., Iowa DNR 2007; Minnesota DNR 2007; Wisconsin DNR 2007). Some of these data have been collected for several years over areas ranging from small wildlife refuges to entire states. Most such data are the results of call surveys conducted from roads or trails, primarily by volunteers according to general standardized protocols (e.g., North American Amphibian Monitoring Program [NAAMP 2007]). While call surveys conducted by volunteers can vary in effectiveness (Crouch and Paton 2002) and produce data of varying or uncertain quality (Genet and Sargent 2003; Shirose et al. 1997; personal observation), ultimately they should produce valuable information regarding the presence of calling amphibian species in the ARMI-MR that can be used as part of a larger integrated effort to describe population statuses (Shirose et al. 1997).

Principal Potential Causes of Declines in the ARMI-MR

We have much more scientific information pertaining to population statuses and their relationships to environmental factors for amphibians than reptiles. Thus, the citations in this section pertain to amphibians, but the issues regarding effects of these environmental factors are also relevant to reptiles and many other organisms.

Habitat Loss

Historical and ongoing habitat loss including fragmentation, agricultural practices, and changes in climate are currently the most pervasive potential threats to the persistence of amphibian populations across large portions of the ARMI-MR (Lannoo 1998; Semlitsch 2000). Enormous quantities of wetland and terrestrial habitat have been lost to development and alteration for agriculture across the Midwest (Lannoo 1998). Such loss undoubtedly has eliminated or reduced many amphibian populations and continues to threaten many others (Hager 1998; Lannoo 1998; Lehtinen et al. 1999; Knutson et al. 1999; Kolozyvary and Swihart 1999; Semlitsch 2000; Semlitsch 2002; Gaggiotti 2003; Hazell 2003; Houlihan and Findlay 2003; Weyrauch and Grubb 2004).

Given the extent to which agriculture has eliminated and fragmented habitat (Tilman et al. 2001), recent economic incentives for converting land to grow crops for biofuels (Westcott 2007; Fargione et al. 2008), the ongoing and widespread development of lands for housing, and the relative lack of large tracts of unaltered, protected landscape in the Midwest, ongoing habitat loss poses a significant threat to amphibian populations in this region. Thus, populations that are able to persist now could become so disconnected from other populations due to habitat loss that the chances of rescue effects in the event of a decline would be limited (Bradford et al. 1993; Lehtinen et al. 1999; Semlitsch 2000; Ovaskainen and Hanski 2003; personal observation).

Agricultural Practices

In addition to agricultural practices resulting in physical elimination and fragmentation of habitats, agricultural use of pesticides and fertilizers poses potential threats to populations via lethal and sublethal mechanisms (Lannoo 1998; Bishop et al. 1999; Marco and Blaustein 1999; Bridges 2000; Bridges and Semlitsch 2000; Semlitsch et al. 2000; Davidson et al. 2001; Marco et al. 2001; Sparling et al. 2001; Kiesecker 2002; Davidson and Knapp 2007; Sparling and Fellers 2007). Evidence suggests that atrazine, for example, can induce abnormal gonadal development in frogs (Hayes et al. 2002a, 2002b, 2003; Carr et al. 2003), as was suggested earlier by Reeder

et al. (1998). Due to the fact that atrazine is applied heavily in the Midwest (National Center for Food and Agriculture Policy 2003; NAWQA 2007; Data Supplement-Figure 2) and is currently the second most heavily used herbicide in the United States behind glyphosate (B. Battaglin, personal communication), these reports are cause for concern with respect to potential effects on amphibian populations in the ARMI-MR (Hayes et al. 2002a, 2002b, 2003).

Atrazine belongs to a class of compounds called triazines, which includes atrazine, cyanazine, metribuzin, prometon, and simazine (University of Florida 2008). These compounds are registered for control of weedy plants, work by inhibiting photosynthesis, and are applied to a variety of crop fields to control the growth of competing vegetation. Triazines are pervasive in the environment and can be persistent, with half-lives exceeding one year in soil and up to 578 days in water under some conditions. They can be moved into surface waters via runoff, spray drift, and atmospheric transport and are detected frequently in rainfall; they also leach into groundwater. These characteristics, together with the level of their use, have contributed to widespread contamination of water that has the potential to be toxic to a variety of aquatic organisms (USEPA 2003; Exttoxnet 2007; PAN-UK 2007).

Of the estimated 76.4 million pounds of atrazine applied annually, use on cornfields accounts for approximately 86%, followed by sorghum at 10%, and sugarcane at 3% (all other uses account for the remaining 1%). Approximately 69-75% of the field corn acreage grown in the United States is treated with atrazine (USEPA 2003; PAN-UK 2007). Increased production of corn for use in biofuels (Westcott 2007) could add to the amount of atrazine applied throughout the Midwest.

While wild organisms living in the large agroecosystem of the Midwest typically are exposed to a mixture of pesticides that includes more compounds than atrazine, effects of atrazine in particular have garnered substantial scientific attention. This is due primarily to the work of Hayes et al. (e.g., 2002a, 2002b, 2003) and others (e.g., Carr et al. 2003) who have demonstrated a range of effects from exposure to atrazine at different concentrations. The collective work of Hayes et al. in particular caused concern because they not only observed that atrazine caused abnormal gonadal development (AGD) in metamorphosed African clawed frogs (*Xenopus laevis*) exposed to 0.1 ppb in the laboratory (2002a), but in separate studies (2002b; 2003) observed that atrazine induced AGD in native leopard frogs in the laboratory at the same concentrations and was associated with AGD in native leopard frogs at several breeding sites in the western and midwestern United States. Hermaphroditism (feminized males) was one of the types of gonadal abnormalities observed in these studies.

These effects are compelling because atrazine concentrations in many of the nation's waters exceed 0.1 ppb. Furthermore, the effects on leopard frogs in the laboratory and in the wild reported by Hayes et al. (2002b; 2003) are noteworthy because they occurred in a native species that is known to have declined in several regions of the United States (e.g., Corn and Fogleman 1984; Lannoo 1998). Atrazine-induced AGD in amphibians had not been identified as a problem of ecological concern until the above studies were conducted. This concern exacerbates others regarding potential indirect effects of atrazine on amphibians via alterations of habitats and food webs (USEPA 2003).

Clear demonstrations of the effects of atrazine on amphibian populations in the wild have not been reported, but hermaphroditism and other forms of AGD are potential mechanisms by which fitness can be reduced directly in individual amphibians. Thus, given the massive and pervasive use of atrazine and other pesticides in the ARMI-MR and elsewhere, the hypothesis follows that these compounds are associated with amphibian declines via AGD and other mechanisms, perhaps on a substantial geographical scale.

We report here on extensive and intensive sampling we conducted for the presence of triazines at amphibian breeding sites in VOYA, the SACN, the UMR, and the NS in 2003 and 2004 to establish baseline information on amphibian exposures to these herbicides.

Pathogens

The potentially lethal amphibian disease, chytridiomycosis, is caused by the fungus, *Batrachochytrium dendrobatidis* (Bd). It has been associated with dramatic declines in 43 amphibian species in seven Latin American countries and 93 species worldwide, including in the United States (Berger et al. 1999; Lips 1999; Bosch et al. 2001; Fellers et al. 2001; Bradley et al. 2002; Weldon et al. 2004; Garner et al. 2005; Herrera et al. 2005; McDonald et al. 2005; Lips et al. 2006; Skerratt et al. 2007). In the western United States and elsewhere in the world, dramatic amphibian die-offs have been associated with Bd in relatively pristine national parks and national forests (Morgan et al. 2007).

The origin of the pathogen is not certain, and the mode by which the disease spreads across the landscape and the mechanism by which it kills amphibians are not well known (Morgan et al. 2007). Typically, susceptible individuals die after Bd has infected their skin, but not all species are similarly susceptible (Rollins-Smith et al. 2003). For example, Bd is the likely proximate cause of dramatic declines of amphibian populations (Lips et al. 2006; Morgan et al. 2007), but African clawed frogs and bullfrogs (*Rana catesbeiana*) are known to carry this pathogen asymptotically (Morgan et al. 2007), and other species appear to be exposed to the pathogen without associated die-offs (Corn 2007; Pearl et al. 2007; personal observations).

There is evidence that the prevalence and virulence of Bd and other diseases can change with temperature (Carey 2000; Kiesecker et al. 2001; Berger et al. 2004; Piotrowski et al. 2004; Zell 2004; McDonald et al. 2005; Pounds et al. 2006) and that anthropogenic changes in environmental conditions in general can cause the emergence of infectious diseases in wildlife (Daszak et al. 2001). Thus, we need to understand if amphibian populations are exposed to this pathogen, the environmental factors that affect its persistence and virulence, and if outbreaks of the disease are causing mortality in the ARMI-MR and elsewhere. Essentially no information has been reported on the presence of Bd and effects of chytridiomycosis in the ARMI-MR. We report here on sampling we conducted in 2006 and 2007 to determine the presence of Bd on amphibian metamorphs at breeding sites in VOYA, the SACN, and the UMR. We also report on the presence of other pathogens among samples of different species collected from sites in these management units from 2002-2005.

Climate Change

Increasing evidence suggests that global warming is and will be associated with intensification of the hydrologic cycle, leading to greater extremes of both floods and droughts in many regions,

including much of the Mississippi River basin (Houghton et al. 1996; Karl and Knight 1998; Groisman et al. 2001; Knox 2000; Milly et al. 2002; Kundzewicz et al. 2007). Rainfall increased in the basin of the Upper Mississippi River over the last 30 years, driving increased runoff and transport of nitrate (and potentially pesticides) into tributaries from agricultural lands (Randall and Mulla 2001; Donner et al. 2002; Donner and Kucharik 2003); presumably this phenomenon extends to other wetlands as well. In addition to changes in precipitation variability, substantial future changes in variation in air temperature also could influence land use directly, resulting in earlier planting of crops and earlier applications of fertilizers and pesticides, for example, and changes in the timing of exposure of amphibians and reptiles to these potentially toxic compounds.

Amphibians require unique regimens of hydroperiod, humidity, and temperature to live and reproduce that make them highly susceptible to climate change (Pounds and Crump 1994; Blaustein et al. 2001). Droughts have been associated with declines of amphibian populations in the Sierra Nevada (Kagarise Sherman and Morton 1993). Changes in climate have been associated with amphibian declines in Costa Rica (Pounds and Crump 1994; Pounds et al. 2006), breeding phenology in Great Britain (Beebee 1995), and the incidence of UVB-induced mortality in the Cascade Mountains of Oregon (Kiesecker et al. 2001). Cold temperatures and insufficient precipitation can result in frozen embryos and breeding sites that dry before metamorphosis can occur, as well as interact with predators and pathogens to increase embryo mortality (Sadinski 2004). Higher frequencies of more extreme conditions could cause substantially greater impacts of climate on recruitment in amphibian populations than have occurred in the recent past. Climate change, among all of the potential universal stressors, has a uniquely powerful potential to affect ecological systems pervasively and, thus, biodiversity that includes amphibians and reptiles, either via directional changes in temperature and precipitation or more frequent extreme events. Monitoring for ecological effects of climate change in the ARMI-MR will be a key component of evaluating statuses of amphibian populations in the near and distant future.

Abnormalities

The most notorious issue pertaining to amphibians in the ARMI-MR has been the relatively high frequencies of frogs with gross physical abnormalities at some locations (Lannoo 1998; Gardiner and Hoppe 1999; Reaser and Johnson 1999; Burkhart et al. 2000; Helgen et al. 2000; Hopkins et al. 2000; Meteyer et al. 2000; Johnson et al. 2001, 2002; Blaustein and Johnson 2003). While often associated with the parasitic trematode, *Ribeiroia ondatrae* (Johnson et al. 2001, 2002; Blaustein and Johnson 2003; Johnson et al. 2007), lines of evidence from the laboratory and field have not been integrated or conclusive regarding the combinations of environmental factors that cause abnormalities consistently in wild populations and to what extent, if any, abnormalities are linked to declines (Ankley et al. 1998; Burkhart et al. 1998; Gardiner and Hoppe 1999; Reaser and Johnson 1999; Dournon et al. 1998; Helgen et al. 2000; Hopkins et al. 2000; Sower et al. 2000; Gilliland et al. 2001; Johnson et al. 2001, 2002; Blaustein and Johnson 2003). This is true despite recent evidence for eutrophication causing increased infection by *R. ondatrae*, a higher frequency of abnormalities, and reduced survival in green frogs (*Rana clamitans*) in mesocosm experiments conducted at Trout Lake, Wisconsin (Johnson et al. 2007).

We know abnormalities are widespread (NARCAM 2007; Blaustein and Johnson 2003), perhaps to some extent because surveys have been conducted more extensively and intensively in recent

years (Blaustein and Johnson 2003). But, while high frequencies of abnormalities have occurred at specific locations, frequencies often are inconsistent at and across sites and high frequencies do not appear to be widespread (NARCAM 2007; D. Green, personal communication), including in the ARMI-MR (Converse et al. 2000; NARCAM 2007; R. Cole, personal communication; D. Green, personal communication; M. Knutson, personal communication; D. Sutherland, personal communication; results reported in this document). Thus, gross physical abnormalities can result from biotic and abiotic factors and get considerable attention in the scientific and popular press, but little evidence so far suggests that they are sufficiently frequent or consistent to cause large-scale population declines. However, they easily could be an indicator of environmental changes (Blaustein and Johnson 2003; Johnson et al. 2007).

A large unknown regarding gross physical abnormalities is how many abnormal larvae die before reaching metamorphosis, the point at which most abnormalities are measured in the field. In addition, the recent work of Johnson et al. (2007) suggests that eutrophication due to agricultural practices could drive a cascade of effects resulting in higher frequencies of abnormalities and reduced survival in amphibians. Given the extent and pervasiveness of agricultural use of fertilizers in the ARMI-MR, this is an issue that warrants further investigation with wild populations living in agricultural landscapes. We report here on the results of our surveys for abnormal metamorphs in VOYA, the SACN, the UMR, and the NS.

Goals and Objectives

Our overarching goals for this work were to begin to describe the presence of amphibian species relative to environmental conditions and stressors on a subset of DOI lands in the ARMI-MR, while providing the NPS and FWS with coincidental inventories of amphibian and reptile species in SACN, VOYA, and MISS, and the NS and UMR, respectively.

Our principal objectives throughout these studies were to: 1) conduct visual-encounter and call surveys for amphibians at potential breeding sites in VOYA, the SACN, the UMR, and the NS and targeted searches for amphibian in the MISS; 2) conduct visual-encounter surveys for reptiles, especially turtles, in the vicinity of amphibian breeding sites in VOYA and the SACN, and to a lesser extent, the MISS; 3) analyze amphibian survey data across management units and years using information theoretic models to estimate species presence and the probability of detection in relation to site-specific and sampling-specific characteristics; 4) collect water samples from amphibian breeding sites in VOYA, the SACN, the UMR, and the NS and analyze them for the presence of triazine herbicides; 5) measure gross physical abnormalities among amphibian metamorphs at breeding sites in VOYA, the SACN, the UMR, and the NS; and 6) sample amphibian populations in VOYA, the SACN, and the UMR for the presence of pathogens, parasites, and symptoms of disease.

Methods

Throughout these studies and immediately after observations were made, we recorded all data into customized data forms (Pendragon Software Corporation; Libertyville, Illinois) loaded into Personal Digital Assistants (PDA) with memory module backups.

We often had to traverse several wetlands on the way to and from the actual wetland we surveyed. We could not carry sufficient quantities of concentrated or dilute bleach solutions to spray hip boots and equipment after walking through each wetland. Therefore, to reduce the possibility of spreading pathogens among sites, we followed a practical approach. We treated hip boots and equipment with a 6-10% bleach solution (DAPTF 2005) before leaving the parking area to boat or walk to each survey site.

Details regarding sampling efforts across management units and years can be found in Data Supplement-Table 13.

Daytime Surveys-Amphibians

Management Units

From 2002-2005 we surveyed amphibian populations in VOYA, the SACN, and the UMR (Figure 2). We surveyed amphibian populations in the NS from 2004-2005 and conducted targeted searches for amphibians in the MISS from 2004-2005 (Figure 2). We surveyed these areas as part of ARMI's objectives for the ARMI-MR and the NPS's objectives for the Inventory and Monitoring Program in the GLKN.

Voyageurs National Park abuts approximately 85 km of the border between Minnesota and Ontario. The SACN is centered along approximately 405 km of the St. Croix and Namekagon Rivers in Wisconsin and Minnesota. The MISS is centered on the upper Mississippi River in Minnesota near Minneapolis. The UMR is centered along approximately 420 km of the Mississippi River floodplain in Iowa, Illinois, Wisconsin, and Minnesota. The NS is located in central Iowa near Des Moines (Figure 2).

VOYA, the SACN, and the MISS are owned and managed to varying extents by the NPS. The UMR and NS are owned and managed to varying extents by the FWS. Geographically these management units lie along a wide north-south axis that encompasses gradients of climate, land use, and habitat types (Figure 2).

Populations Sampled and Areas of Inference

The populations we sampled were almost exclusively amphibian subpopulations that reproduce in lentic wetlands.

Due to logistical constraints accessing and traversing the interior of VOYA, our pre-determined area of inference for VOYA was all of the area within one kilometer of roads, trails, or boat access. The entire NPS-managed portion of the SACN was our area of inference for the SACN, as was the entire FWS-managed portion of the UMR for the UMR. All of the NS was included in the area of inference for the NS. We conducted targeted searches at specific locations in the



Figure 2. The five management units we surveyed relative to the Midwest Region of USGS's Amphibian Research and Monitoring Initiative and land cover (USGS's 1998 Global Land Cover Characterization Project). The pink areas represent mostly cropland, primarily corn.

MISS in an attempt to confirm the presence of select species. Thus, the results of our MISS work apply only to those specific MISS locations.

Site Selection

Our method of selecting sites for daytime amphibian sampling generally was consistent across VOYA, the SACN, the UMR, and the NS. We used a geographic information system (GIS) to create a grid of 25-ha square cells (500 m on a side) over the area of inference in each individual management unit (Figure 3).

We did not further divide areas in VOYA. For the SACN, we divided the approximately 405 km of the park into 12 equal, contiguous linear sections and randomly selected a subset of eight sections to sample (Figure 4). We blocked the UMR according to the 14 pools created by the pre-existing lock and dam system in the refuge and selected six pools randomly (4, 7, 8, 10, 13, and 14) to sample. We divided the NS into three equal sections, all of which we sampled.

We randomly selected individual cells to sample within each management unit from the cells available in each grid for that area and proceeded to sample them once we confirmed they contained at least one wetland that could be used by at least one local amphibian species for breeding. We did this by using global positioning system (GPS) receivers to census each cell and locate all wetlands within it. We then evaluated whether any such wetlands could support amphibian breeding and reproduction based upon size, likely hydroperiod, depth, vegetation, and

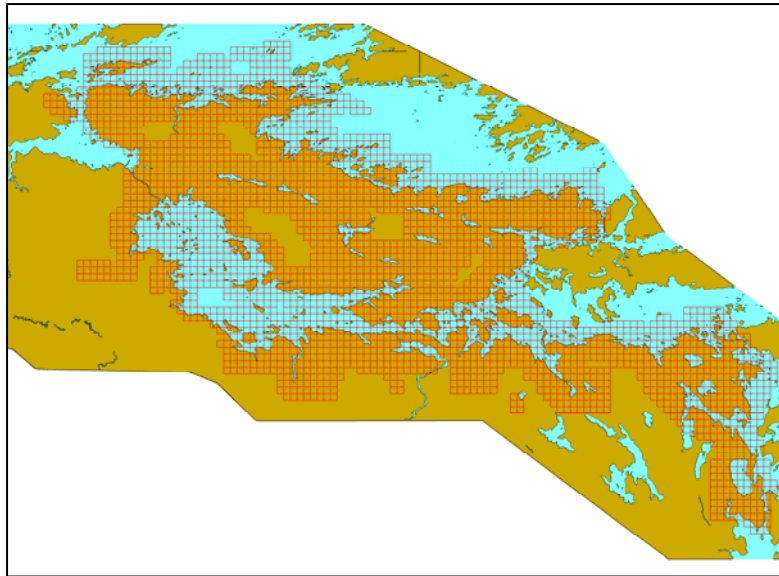


Figure 3. Voyageurs National Park with a grid of 25-ha cells superimposed over the area of inference (all of the area within 1 km of roads, trails, and boat access).

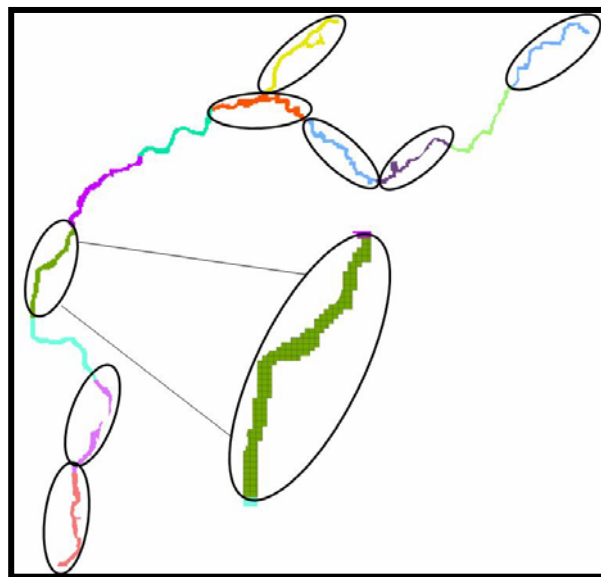


Figure 4. The eight sections we sampled for amphibians and reptiles in the St. Croix National Scenic Riverway.

our professional experience. Most wetlands we located qualified as potential amphibian breeding habitat. We repeated the process of identifying cells containing at least one potential breeding site until we had a sufficient number of cells per management unit. We discarded any cells which contained greater than 50% private land or 75% contiguous open water. Ultimately, we identified 24 cells to sample in VOYA and a minimum of six individual cells to sample in each of the sections in the SACN and NS and each of the pools in the UMR.

Sampling Frequency

We tried to sample most potential breeding sites once during each of the early (late March-early May), mid (mid May-late June), and later (late June-early August) portions of the breeding season for resident amphibians in each management unit. This enabled us to observe the presence of various life stages of all species that might breed in a wetland over the course of the season. Due to wetlands drying, time constraints, and other unforeseen circumstances, we were not able to sample all sites on three separate occasions in each year. Sampling at each site on each occasion was conducted by two persons working independently but according to the same protocols. Each person's sampling effort on each occasion was treated as an independent sampling event for that site.

Environmental Data

Site Characterizations: We measured a set of descriptive variables pertaining to site characteristics and conditions during sampling. We measured GPS coordinates for each site, type of wetland, and size of the wetland basin once for each site the first time we sampled it. We measured all other variables for each site on each sampling occasion.

Whenever possible, we used a laser range finder to measure lengths and widths of wetland basins. On some occasions when we could not determine the boundaries of the wetland due to large size or insufficient objects along the edge to reflect the rangefinder's beam, we estimated with the naked eye. We also estimated the percent of the wetland basin that had canopy cover and the percent of standing water that contained emergent vegetation using the naked eye. We described the type or species of dominant emergent vegetation based upon keys for each area.

Weather Conditions: We measured air temperature, wind speed, and relative humidity with Kestrel 3000 or 4000 pocket weather meters (Nielsen-Kellerman Co. Boothwyn, PA).

Barometric pressure and the trend in barometric pressure during the last two hours were measured using a Brunton Sherpa weather meter (Brunton, Inc., Riverton, WY). These measurements were taken at the edge of the wetland after instruments had acclimated for a minimum of ten minutes approximately two meters off the ground. Percent cloud cover and standardized categories of precipitation events were described via the naked eye.

Water pH and Conductivity *In Situ*: We measured the pHs, conductivities, and water temperatures of potential breeding sites *in situ* one time at one location during each sampling occasion using either Hanna (Woonsocket, Rhode Island) Model HI991300 portable waterproof field meters or Oakton (Vernon Hills, Illinois) Model PC10 portable waterproof field meters. We calibrated meters according to the manufacturer's recommendations using appropriate standards and deionized water. We typically measured pHs, conductivities, and water temperatures coincidentally in approximately 20-40 cm of water.

Pesticides-Sampling and LCMS Analyses in Kansas: Our ARMI partners from USGS's Water Resources Discipline (WRD) in Mounds View, Minnesota, collected water samples in 2003, 2004, and 2005 for analyses at the USGS's WRD Organic Geochemistry Research Group in Lawrence, Kansas. All water samples for pesticide testing were filtered at the collection site using glass-fiber filters with nominal 0.7- μ m pore diameter to remove suspended particulate matter. Sampling equipment, filtering procedures, and sample analyses were in accordance with

WRD standards and specifications. Liquid chromatography/mass spectrometry (LCMS) was used to analyze samples for pesticides. Details on the relevant methods and procedures used at the Kansas laboratory are described in Lee et al. (2002).

Triazine Herbicides—Sampling and ELISA Analyses in Wisconsin: We collected water samples at potential breeding sites we sampled for amphibians from 2003-2005. We analyzed these samples at the USGS's Upper Midwest Environmental Sciences Center in La Crosse, Wisconsin, via Enzyme-linked Immunosorbent Assays (ELISA) purchased as kits from Abraxis Ltd. (Warminster, Pennsylvania). We collected unfiltered water samples from just below the water's surface at one shallow location per site. Sterile sampling bottles were filled and capped while the bottle was completely submerged by a person wearing latex gloves. We conducted analyses according to the manufacturer's recommended protocols using supplied antibodies designed to react specifically with atrazine and some related triazines (Abraxis 2007).

Call Surveys

We conducted call surveys during each daytime visit to a potential breeding site. Call surveys were conducted by naked ear for ten minutes from a location ten to 30 meters away from the wetland, hidden from view when possible to reduce the likelihood of interrupting calling or breeding. We began surveying after waiting five minutes to allow any interrupted individual amphibians to resume calling. We used an independent double-observer approach in these surveys, which involved two observers recording data independently of each other without communication. Each surveyor's results were treated as a separate sample.

Visual-encounter Surveys

We conducted time-constrained visual-encounter surveys (VES) during each sampling occasion using the naked eye and dip nets. These surveys were conducted immediately after call surveys and measurements of site and weather conditions were completed. We walked slowly along a haphazardly chosen transect throughout the potential amphibian habitat in each wetland for 20 minutes. We surveyed for all life stages of all possible species during the VES. One sweep with a dip net was taken at each of 20 nodes, each two-minutes walking time apart along each transect. Survey time was suspended (stopwatches) while results of sweeps were assessed and recorded. Amphibians encountered while walking between nodes not identifiable with the naked eye *in situ* were caught with dip nets. Two independent VES were conducted along separate transects at each site per sampling visit, one by each of two observers, if the size of the wetland permitted.

Metamorph Abnormality Surveys

We conducted time-constrained surveys for abnormal metamorphs of several species after completing the VES when metamorphs were likely to be present. We caught as many recent metamorphs as possible (tails fully resorbed) during 20-minute sampling periods and evaluated all individuals for several gross physical abnormalities afterwards. Surveys were conducted in appropriate habitat near the wetland where recent metamorphs were likely to be present. Types of abnormalities were categorized and defined according to Meteyer et al. (2000). Typically an independent metamorph survey was conducted by each of two surveyors per site per sampling visit. This was not always the case, as when animals were particularly difficult to catch due to vegetation or when the number of metamorphs or size of the habitat was insufficient. We only conducted surveys when we estimated species were metamorphosing given we were not

monitoring larval development closely. Therefore, the timing of our surveys was not necessarily well-aligned with actual timing of metamorphosis. In addition, environmental conditions, such as hydroperiod, greatly affected the timing of actual metamorphosis and the presence of metamorphs during surveys.

Collections for General Health Screening

We collected healthy appearing live specimens for general health screening and sent them to Dr. Green at the USGS National Wildlife Health Center (NWHC) in Madison, Wisconsin, for examination according to standard NWHC protocols. We also sent some individuals with obvious abnormalities or unhealthy appearances to Dr. Green for examination. All specimens were sacrificed and examined for diseases, viruses, bacteria, fungi, parasites, and abnormalities of the skeleton and reproductive organs. Collection and handling of specimens, shipping procedures, euthanizations, and disposal of specimens were conducted in accordance with NWHC standards and protocols.

Sampling and Analyses for *Batrachochytrium dendrobatidis*

In 2006 (25 total sites) and 2007 (44 total sites), we sampled recently metamorphosed amphibians for Bd at known breeding sites in VOYA (nine sites in 2006, 13 sites in 2007), the SACN (eight sites in 2006, 13 sites in 2007), and UMR (eight sites in 2006, 18 sites in 2007). We also sampled four non-DOI sites in Wisconsin in 2006. We sampled some sites in both years, but there were 56 unique sites in total (Data Supplement-Tables 27-30). We typically sampled 20 apparently healthy metamorphs of each species available per site during visits to sample for Bd in 2006. In 2007, we typically sampled 30 such metamorphs during sampling for Bd. We used clean techniques to avoid cross contamination of captured metamorphs and cotton-tipped swabs (Puritan brand, 15 cm, no-glue cleaning sticks) to swab each animal a minimum of 30 times across the ventral skin on the thighs, abdomen, and hind limbs, including the interdigital webbing (Livo 2004). We released all animals where they were collected immediately after processing. To reduce analytical costs while maintaining our chances of detecting the presence of Bd, we typically pooled swabs from ten individuals of the same species from the same site in one sterile vial containing 70% EtOH, which we then sealed and sent to Pisces Molecular LLC in Boulder, Colorado, for polymerase chain reaction (PCR) analyses. Methods used by Pisces Molecular LLC are described in Annis et al. (2004).

Data Analyses

We conducted daytime call and visual-encounter surveys to document the presence of amphibians at breeding sites. Naïve occupancy estimates (number of sites searched where species *x* was observed/number of sites searched) were determined for all species observed.

We also analyzed combined data from all daytime amphibian surveys conducted in VOYA, the SACN, the UMR, and the NS from 2002-2005 via information-theoretic models that MacKenzie and Kendall (2002), MacKenzie and Nichols (2004), and MacKenzie et al. (2002, 2003, 2006) developed and incorporated into the computer program, PRESENCE (<http://www.mbr-pwrc.usgs.gov/software.html>).

Two strengths of using MacKenzie et al.'s models are: 1) they allow for calculating detection probabilities based upon repeated sampling and therefore provide a more accurate estimate of the

true occupancy (Schmidt 2003; Royle 2004; Pellet and Schmidt 2005), and 2) measures of variables describing ambient environmental conditions can be used as covariates in the models (e.g., Pellet and Schmidt 2005). This latter feature enables the analyst to investigate the influence of environmental variables on the occupancies and detection probabilities of species sampled.

Another important aspect of this method is that it was developed in collaboration with scientists from ARMI and is used as the primary analytical tool for data collected under ARMI throughout the United States. Consequently, use of the occupancy metric has been adopted by other NPS Inventory and Monitoring Networks and also is likely to be the analytical method of choice for the Great Lakes National Program Office's Lake Superior Basin Herpetological Program (Hecnar and Casper, personal communication). Thus, the NPS will be able to compare estimated occupancies and detection probabilities calculated for populations in this report with those calculated for populations regionally and nationally.

We conducted a multi-year analysis on the aforementioned daytime survey data via PRESENCE. We analyzed data for eight species which we sampled sufficiently to satisfy occupancy-analysis/PRESENCE requirements for numbers of sites and repeat surveys from 2002-2005. We did not include any species which were rare or seldom detected in our occupancy analysis. Based upon our experience and published reports, we hypothesized *a priori* which factors likely could influence the presence and detection of our study species and tested those variables in our occupancy models. The site-specific covariates we selected *a priori* were management unit (MAN = VOYA, SACN, UMR, or NS), surface area of the breeding site (in ha), and wetland type (according to standardized categories). *A priori* sampling-specific covariates we tested in the models were date (day, month, year), time of day, and observer (in terms of relative experience). These covariates potentially had direct bearing upon habitat where an amphibian species could live and/or whether we could detect it if it was present.

Nighttime Call Surveys

Management Units

We conducted nighttime call surveys in VOYA, the SACN, the UMR, and the NS from 2002-2005 and the MISS during 2004 and 2005.

Populations Sampled and Areas of Inference

We sampled breeding subpopulations of calling anurans within the presumed area of inference. We primarily used parabolic microphones to conduct nighttime call surveys from listening locations along roads to complement our efforts during the day. From 2002-2005 in VOYA, and from 2002-2004 in the SACN and the UMR, these locations were picked non-randomly to be near wetlands we sampled during the day (see next section). From 2004-2005, we did the same for the NS. Thus, we cannot extrapolate results from those years in those management units beyond those specific sampling locations.

In 2005, we selected individual sites randomly from a pool of potential sites along roads in the SACN and the UMR (see next section). Thus, we can extrapolate our results for these two areas during 2005 and 2006 to all of the area encompassed within the range of the parabolic microphones at all possible listening locations in the entire pool of possible listening locations

from which we selected randomly. Given that the range of the parabolic microphones varies with land cover, topography, and weather, the specific boundaries of these areas of inference are not clear.

Site Selection

As defined in the previous section, from 2002-2005 in VOYA and from 2002-2004 in the SACN and the UMR, we purposely located nighttime call-survey sites near wetlands we sampled during the day, as conducting night surveys at the daytime sites was not possible logistically. These nighttime survey sites largely were located as eight nodes spaced approximately 200 meters apart along a linear transect. We located at least one such transect in each of the sections or pools in the SACN, the UMR, and the NS. We were greatly constrained by road availability relative to daytime sites in VOYA, where we placed four transects in the only areas possible, one along the northwestern portion of the park and three along the southwestern portion.

Two hundred meters between nodes along a transect did not allow independence among observations collected with a parabolic microphone. Thus, we treated each of these transects of individual listening nodes as one site for the purpose of reporting proportions of sites where species *x* was heard at night via our use of this approach (Table 7).

In 2005, we established a set of individual listening locations selected randomly from a pool of possible listening locations along roads within the SACN and the UMR. We used a GIS to identify all possible listening locations at least 1000 m apart (to reduce the possibility of double sampling an area with the parabolic microphones from different listening locations) along roads in each management unit. All these possible locations served as the pool of locations from which we then selected four actual listening locations randomly within each section or pool of the SACN and UMR, respectively. We also added one non-random listening location to the set of randomly selected listening locations in each section or pool, the middle listening location from the aforementioned transects we surveyed in previous years.

Sampling Frequency

From 2002-2004, and for 2005 in VOYA, we typically sampled three times from each listening location over the course of the amphibian reproduction season in each management unit, once each during early, mid, and later segments of the season. We coordinated the timing of nighttime surveys occur around the same dates daytime surveys were conducted nearby. One observer sampled one complete transect per night.

In 2005 in the UMR and SACN, one of two observers working independently sampled from each listening location (sequences selected randomly) up to three times within each of three two-week intervals over the course of the amphibian reproduction season, for a maximum total of nine repeat surveys per location. The three intervals occurred during the early, mid, and late portions of the season. Due to unforeseen circumstances, we did not always manage nine repeat surveys at all listening locations in each management unit.

Environmental Data

Site Characterizations: We recorded the GPS coordinates for each listening location during our first survey from each location.

Weather Conditions: We measured the same weather variables described for daytime sampling using the same equipment at night. For transects, we recorded weather data at the first and last listening location. Measurements were taken outside of vehicles after the instruments had acclimated for a minimum of ten minutes. We recorded weather data at each listening location in the SACN and the UMR during 2005.

Call Surveys

We typically began nighttime call surveys approximately 30-60 minutes after sunset and continued until we completed the pre-determined set of survey sites for each night. We almost always sampled amphibians calling at night while listening through headphones (AKG Acoustics Model K240; Vienna, Austria) plugged into a Telinga (Tobo, Sweden) parabolic stereo or directional microphone, which was powered by a HHB (London, England) Portadisc MDP500 Recorder. We surveyed by naked ear when sampling conditions did not allow the use of parabolic microphones, such as lightning, heavy rain, or high winds.

We conducted call surveys with parabolic microphones for a period of ten minutes from inside a vehicle parked at listening locations with the parabolic microphone extended outside and above the roof of the vehicle. We stood outside of and away from vehicles while conducting naked-ear surveys. We began surveys at least five minutes after arriving at a site to allow any calling amphibians we disturbed to resume calling. We quantified the number or category of calling individuals we heard during each survey by species and the distance and direction from which calls originated.

Data Analyses

Before 2005, we used nighttime call surveys primarily to complement our observations of presence during daytime surveys and did not analyze data beyond describing the proportions of sites from which we heard calling species. Nor have we analyzed data we collected in the SACN and the UMR in 2005 beyond simple proportions because we changed listening locations again in 2006 and do not expect to incorporate the 2005 data into a long-term monitoring data set at this time.

Targeted Searches and Cover Objects–Amphibians and Reptiles

Management Units

We conducted basking turtle surveys and surveys of natural cover objects during the day in VOYA and the SACN from 2002-2004 in or near the same cells in which we conducted daytime surveys. We also conducted targeted searches in the MISS during 2004 and 2005. We placed artificial cover objects within and near sampling cells in VOYA and the SACN in 2003 and sampled them from 2003-2005.

Populations Sampled and Areas of Inference

Generally, we sampled turtle populations that basked and amphibian, snake, and lizard populations that dwelled under natural and artificial cover objects within or near our sampling cells in VOYA and the SACN. Our areas of inference were similar to those described for these management units in the methods section on daytime amphibian surveys.

During targeted searches in the MISS, we sampled portions of amphibians and reptile populations that existed in the specific locations we surveyed. We cannot extrapolate our results from these searches to any larger portion of the MISS.

Site Selection

We surveyed amphibian sampling sites for basking (or otherwise observable) turtles in VOYA and the SACN and at other appropriate sites in or near the 25-ha daytime survey cells, such as logs or rocks in rivers, creeks, or lakes.

We randomly selected 18 25-ha daytime sampling cells in VOYA and 20 such cells in the SACN to sample via artificial cover boards. We used a GIS to randomly select coordinates for placement of one cover board within each of these cells. We also used the same process to select coordinates for placement of artificial cover boards outside of any previously sampled cells, but within 100 m of road access. We deployed 18 additional artificial cover boards at such locations in VOYA and 35 in the SACN. We did not place these cover boards within five meters of water, on slopes greater than 35°, or on bare rock.

We selected sites to survey during targeted searches in the MISS based upon habitat requirements and life-history characteristics of the species we targeted. We used a GIS of land cover and vegetation data layers, as well as topographical maps, to identify potential survey sites. We also based site selections on discussions with local experts and field guides.

Sampling Frequency

From 2002-2004, we conducted basking turtle surveys and surveyed under natural cover objects as part of sampling potential amphibian breeding sites according to the methods and frequencies described for daytime amphibian surveys. We also sampled artificial cover objects during the same three potential sampling occasions from 2004-2005.

We conducted targeted searches in the MISS during each of three sampling occasions in 2004, at monthly intervals beginning in May. We also attempted to sample during April of 2004, but could not complete the requisite coordination with all the different local municipalities and county and state agencies that have jurisdiction over portions of the MISS and only managed very limited surveys during that visit. Sampling occasions ranged from two to seven days. We also conducted targeted searches in the MISS during April of 2005, mostly for calling anurans.

Basking Turtle Surveys

Basking turtle surveys consisted of time-constrained visual searches using binoculars and spotting scopes (Casper 2004). We conducted these surveys for ten minutes from a single observation point approximately ten to 30 meters away from basking objects at each survey site.

Natural Cover Objects

From 2002-2004, we surveyed under at least ten natural cover objects in each 25-ha cell we sampled via daytime amphibian surveys in VOYA and the MISS. Natural cover objects included any object which may have provided adequate refuge for amphibian or reptile species, including downed timber, rocks, and human debris.

Artificial Cover Objects

Artificial cover objects consisted of an array of four 4 x 28 x 61 cm pieces of untreated lumber. We placed the four pieces of lumber within each array side to side on the long side and propped up the fourth piece on top of the edge of the third piece. We anchored each array with small posts we pounded into the ground. The axis of each array was aligned east to west to maximize solar exposure.

Targeted Searches

We conducted visual-encounter surveys for species we targeted in the MISS. These included dip-net surveys in wetlands, surveying potential basking locations with binoculars, spotting scopes, and the naked eye, and surveying under natural cover objects. We conducted nighttime call surveys according to the methods described earlier. After we observed a targeted species, we no longer surveyed for that species.

In a limited attempt to determine the presence of common mudpuppies (*Necturus maculosus*), we deployed aquatic funnel traps in the SACN in 2005 and the MISS in 2004. We deployed five traps for five nights each in the MISS and three traps for one night in the SACN in riverine habitat appropriate for this species. Traps deployed in the MISS were baited with commercially available earthworms and minnows. Traps deployed in the SACN were not baited. We did not deploy traps beyond one night in the SACN because we caught a mudpuppy the first night.

Results

Daytime Surveys-Amphibians

Environmental Data

Site Characterizations: The characteristics of potential aquatic amphibian breeding sites varied considerably within and among management units (Data Supplement-Tables 1-5). Wetland types in VOYA and the SACN were representative of those expected in the northern forested uplands of the Midwest, ranging from temporary forested and shallow vegetated wetlands to beaver impoundments. While we did find potential aquatic amphibian breeding habitat along non-flowing backwaters of the Namekagon and St. Croix Rivers in the SACN, most appropriate habitat existed in small lentic wetlands largely isolated from flowing water (not considering the habitat of common mudpuppies in this case). Likewise, we found aquatic amphibian breeding habitat in shallow areas of some bays and relatively protected areas of Rainy and Kabetogama Lakes in VOYA, but most breeding habitat was in relatively small, shallow wetlands upland of lakes.

Aquatic amphibian breeding habitat existed among various types of wetlands in the UMR (Figure 5; Data Supplement-Figure 3; Data Supplement-Tables 1-5), including in shallow water along sheltered edges and backwaters of channels and sloughs with non-flowing water. While open water (Data Supplement-Figure 3) as channels and sloughs dominated floodplain wetlands

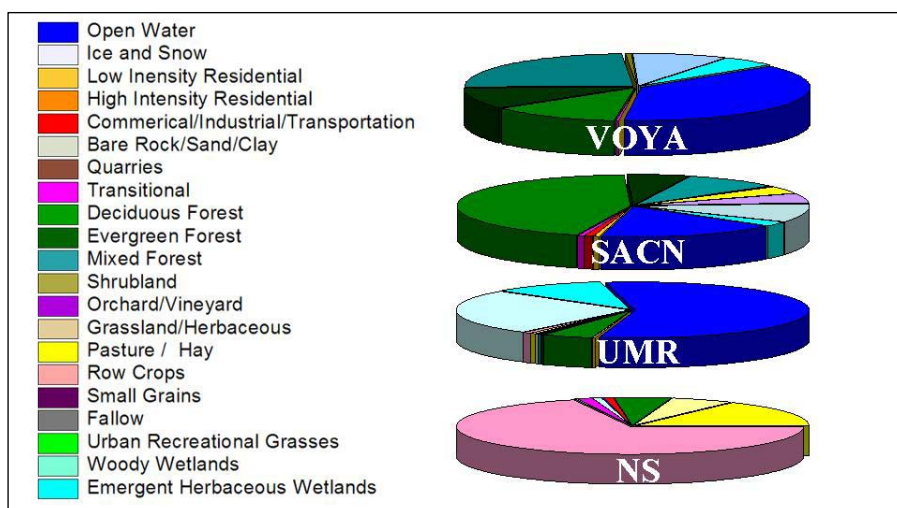


Figure 5. Land-cover classes by management unit based upon the 2000 National Land Cover Data set. NS = the Neal Smith National Wildlife Refuge; UMR = the Upper Mississippi National Wildlife and Fish Refuge; SACN = the St. Croix National Scenic Riverway; VOYA = Voyageurs National Park.

along the Mississippi River, considerable forested and non-forested upland habitat contained many small, shallow, often ephemeral wetlands in which amphibians did, or potentially could,

breed. Floodplain forests dominated the upland portion of the floodplain areas we surveyed (Figure 5). Wetland types embedded in these forests varied considerably in size, depth, and vegetation.

The NS is embedded in the agricultural landscape of central Iowa. This area was once a part of a vast prairie landscape in the Midwest. Wetland types in this refuge were the most homogenous (Data Supplement-Tables 1-5), although amphibians bred in everything from former stock ponds to tire ruts. A substantial portion of the diversity of aquatic amphibian breeding sites at the NS existed as former stock ponds, which were the only or primary breeding sites for species such as eastern tiger salamanders (*Ambystoma tigrinum*), leopard frogs (a potential hybrid zone between northern leopard [*R. pipiens*] and plains leopard frogs [*R. blairi*]), and bullfrogs. The FWS was eliminating these stock ponds as part of their efforts to restore hydrological linkages at the NS.

Water pH and Conductivity *In Situ*: Based upon measurements *in situ*, pHs were highest in the NS and the UMR and most acidic in VOYA (Figure 6; Data Supplement-Tables 1-5). Conductivities in the NS and UMR were higher than in the SACN and VOYA (Figure 7; Data Supplement-Tables 1-5).

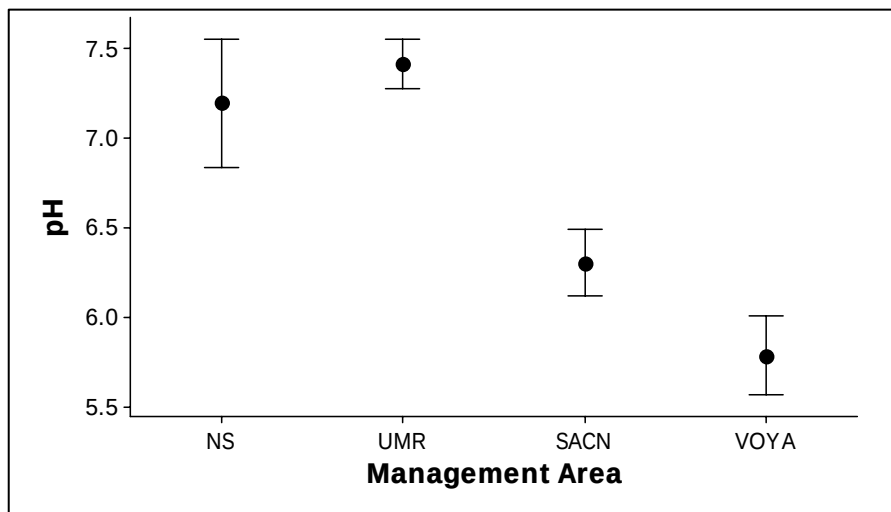


Figure 6. Mean pHs of potential amphibian breeding sites across years (+/- 95% confidence intervals).

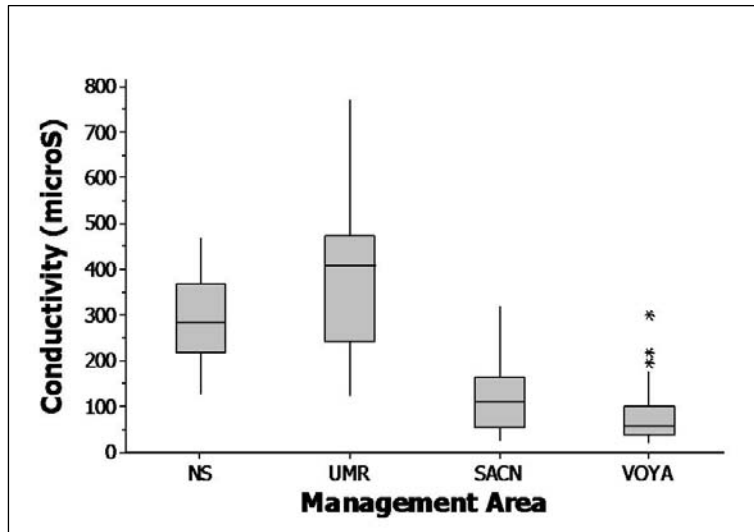


Figure 7. Conductivities of potential amphibian breeding sites across years. Lines within the boxes indicate median values. The middle 50% of the data are contained within each box. Stars (*) indicate outliers.

Triazine Herbicides: Based upon a comparison of results from ELISA and LCMS analyses conducted on the same 13 samples collected in 2004 and the same 12 samples collected in 2005 from breeding sites in the UMR (Data Supplement-Tables 6-11), we learned the following:

1. Filtering samples did not change triazine concentrations significantly (Figure 8).
2. LCMS analyses confirmed the presence of atrazine and atrazine breakdown products in our samples, and the atrazine parent compound was dominant among all possible compounds (Data Supplement-Tables 6 and 7).
3. ELISA results likely indicated mostly atrazine and atrazine breakdown compounds among triazines detectable with this ELISA method, given LCMS analyses showed other triazines in our samples often were below detection limits, with some exceptions (Data Supplement-Tables 6-11).
4. Triazine concentrations obtained from ELISA tended to be higher than atrazine concentrations from LCMS, but trends by both methods were similar (Data Supplement-Tables 6-11).

We sampled 99, 152, and 89 individual breeding sites for triazines across management units during 2003, 2004, and 2005, respectively (Data Supplement-Tables 8-11). With few exceptions, we did not sample sites more than once during 2003. We do not address 2003 data further in this report, although they are listed in Data Supplement-Tables 8-11. Based upon our ELISA results, triazine concentrations varied considerably among individual sites within management units during 2004 and 2005, but breeding sites in the UMR and NS as a whole generally had higher concentrations of triazines than SACN, which in turn had higher concentrations than VOYA (Figure 9; Data Supplement-Figure 4; Data Supplement-Tables 8-11). Of the 523 (45-NS, 270-UMR, 94-SACN, 114-VOYA) water samples contained in this 2004-2005 data set, including

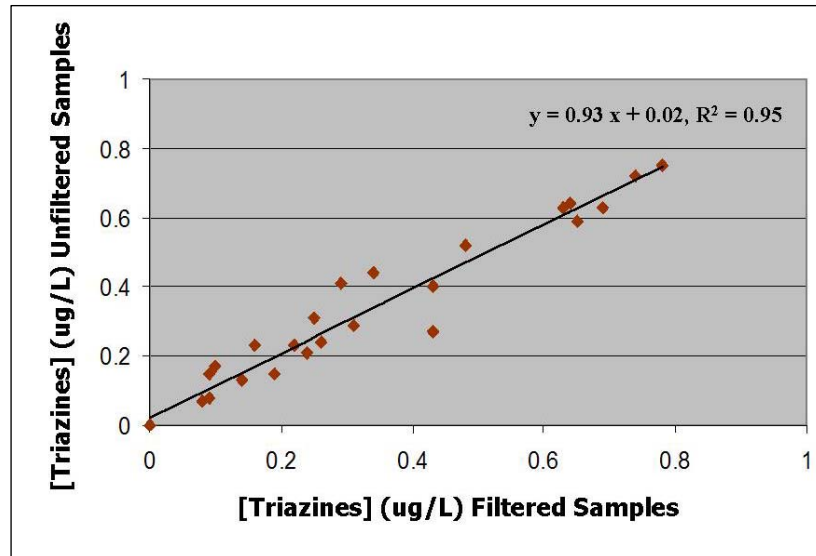


Figure 8. Triazine concentrations in filtered versus unfiltered water samples collected from the same breeding sites and times over several sites and sampling occasions in the Upper Mississippi River National Wildlife and Fish Refuge in 2004 (13 samples) and 2005 (12 samples).

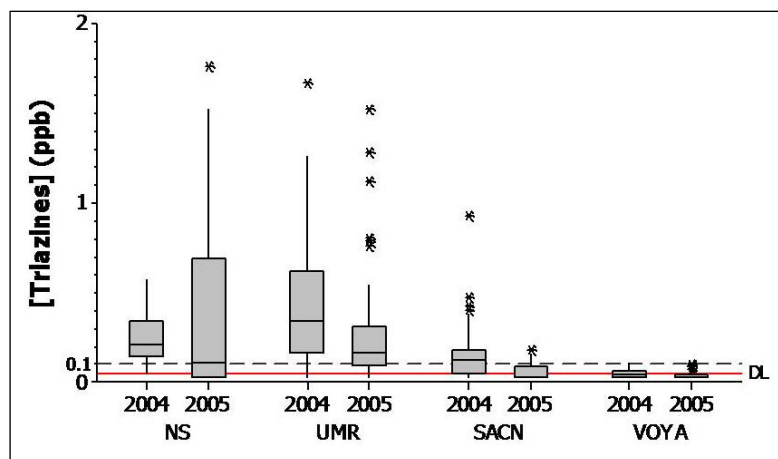


Figure 9. Triazine concentrations in water samples from potential amphibian breeding sites across management units in 2004 and 2005. A concentration of 0.1 ppb induced abnormal gonads in leopard frogs (Hayes et al. 2003). Some of the high outlier values in the NS and the UMR are excluded to better illustrate comparisons across management areas. DL = the detection limit for the ELISA.

multiple samples from the same sites, 161 (30.8%) mean triazine concentrations (of two laboratory replicates of one water sample from the field) were below the detection limit of 0.050 ppb, mostly in the SACN and VOYA.

Fifty-five percent of the values above the detection limit also were above 0.1 ppb (Data Supplement-Figure 5), which is the concentration that induces abnormal gonads in leopard frogs in the laboratory (Hayes et al. 2003). Most of those samples came from the NS and UMR. These relative concentrations follow the physical proximity of each of these management units to extensive areas of row crops (Figure 2; Data Supplement-Figure 6) where triazines are applied in large quantities (Data Supplement-Figure 2; Data Supplement-Tables 8-11).

Based upon our more intensive sampling of breeding sites in the UMR during 2004, triazine concentrations at breeding sites and in the main channel of the Mississippi River fluctuated in relation to water levels in the main channel (Figure 10). This reflected the extent of hydrological connectivity between the slightly elevated breeding sites and the river's channels (personal observations). Triazine concentrations tended to increase when these more upland breeding sites were connected to the river's channels via high water.

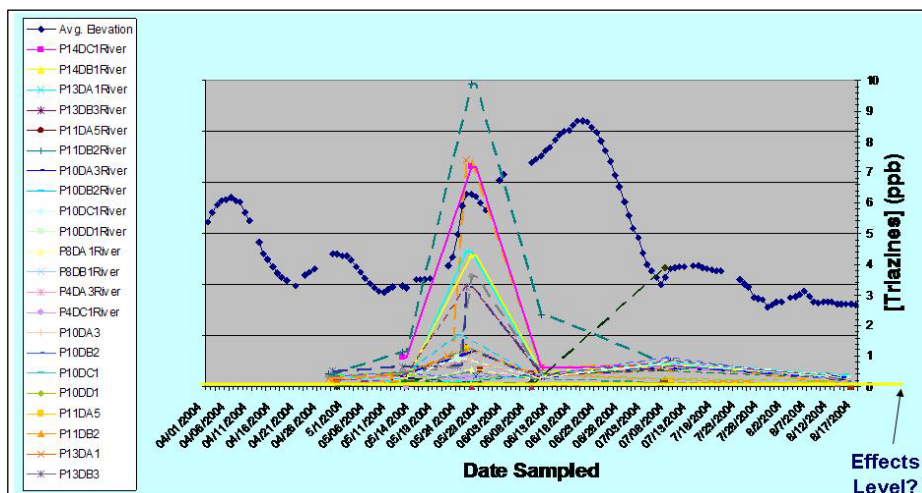


Figure 10. Triazine concentrations for amphibian breeding sites in the Mississippi River floodplain (P = pool) and for additional sampling sites in the main river channel (River in the site name) from late April to early August of 2004. The dark blue beaded line is the hydrograph of the Mississippi River. Effects level (0.10 ppb) = the atrazine concentration that caused abnormal frog gonads in laboratory experiments (Hayes et al. 2003).

Other Pesticides: We collected one water sample from each of a small number of breeding sites from 2003-2005 for herbicide analyses via LCMS; these sites were primarily in the UMR (Data Supplement-Tables 6 and 7). While atrazine was detected consistently across sites and years in

the UMR, it was present only at one site sampled in VOYA during 2003 and none in the SACN (Data Supplement-Tables 6 and 7). We found none of the other pesticides for which we analyzed except acetochlor, alachlor, and metolachlor, which were present at one breeding site in the UMR. We did not conduct such sampling and analyses for any other breeding sites and these simply were intended to help inform and complement the results obtained via our ELISA analyses for triazines

Call and Visual-encounter Surveys

Species Observed: We developed lists of species potentially present in each management unit to compare with our field observations. Those lists were based upon species-specific information provided by biologists from the NPS and FWS (Tables 1 and 2) and information we obtained from field guides (e.g., Conant and Collins 1998) and the ARMI Amphibian Distribution Atlas (ARMI 2007). Species lists for FWS units are provided in Data Supplement-Table 12.

Based upon daytime, nighttime, and targeted surveys, we found 71, 84, 72, and 75% of the amphibian species potentially present at VOYA, the SACN, the UMR, and the NS, respectively (Figure 11, Table 1; Data Supplement-Table 12). We did not target individual species in VOYA, the SACN, the UMR, and the NS, but sampled potential breeding sites randomly within pre-determined areas of inference. We did target species separately in the MISS and describe those results at the end of this section. Surveys we conducted and species we observed at each site are listed by management unit in Data Supplement-Tables 13-21.

Table 1. Potentially present amphibian species listed by the National Park Service Inventory and Monitoring Program and amphibian species observed by ARMI–MR for the Mississippi National River and Recreation Area (MISS), the St. Croix National Scenic Riverway (SACN), and Voyageurs National Park (VOYA).

Common Name	Scientific Name	Management Unit where confirmed or potentially present					
		MISS		SACN		VOYA	
		Potentially Present	Observed	Potentially Present	Observed	Potentially Present	Observed
blue-spotted salamander	<i>Ambystoma laterale</i>	X	-	X	X	X	X
spotted salamander	<i>Ambystoma maculatum</i>	X	-	X	X	-	-
eastern tiger salamander	<i>Ambystoma tigrinum tigrinum</i>	X	X	X	-	-	-
central newt	<i>Notophthalmus viridescens louisianensis</i>	X	-	X	X	X	X
four-toed salamander	<i>Hemidactylium scutatum</i>	X	-	X	X	-	-
eastern red-backed salamander	<i>Plethodon cinereus</i>	X	-	X	X	X	-
common mudpuppy	<i>Necturus maculosus maculosus</i>	X	-	X	X	X	-
eastern American toad	<i>Bufo americanus americanus</i>	X	X	X	X	X	X
Canadian toad	<i>Bufo hemiophrys</i>		-	-	-	X	-
Cope's gray treefrog	<i>Hyla chrysoscelis</i>	X	-	X	X	X	-
eastern gray treefrog	<i>Hyla versicolor</i>	X	X	X	X	X	X
northern spring peeper	<i>Pseudacris crucifer crucifer</i>	X	-	X	X	X	X
boreal chorus frog	<i>Pseudacris maculata</i>	X	X ¹	X	X ¹	X	X
western chorus frog	<i>Pseudacris triseriata</i>	X	- ¹	X	- ¹	-	-
American bullfrog	<i>Rana catesbeiana</i>	X	X	X	X	-	-
northern green frog	<i>Rana clamitans melanota</i>	X	X	X	X	X	X
northern leopard frog	<i>Rana pipiens</i>	X	X	X	X	X	X
pickerel frog	<i>Rana palustris</i>	X	-	X	-	-	-
mink frog	<i>Rana septentrionalis</i>	X	-	X	X	X	X
wood frog	<i>Rana sylvatica</i>	X	-	X	X	X	X

¹We did not differentiate between the boreal chorus frog and the western chorus frog, the two species overlapped theoretically in the MISS and SACN.

Table 2. Potentially present reptile species listed by the National Park Service Inventory and Monitoring Program and reptile species observed by ARMI–MR for the Mississippi National River and Recreation Area (MISS), the St. Croix National Scenic Riverway (SACN), and Voyageurs National Park (VOYA).

Common Name	Scientific Name	Management Unit where confirmed or potentially present					
		MISS		SACN		VOYA	
		Potentially Present	Observed	Potentially Present	Observed	Potentially Present	Observed
eastern snapping turtle	<i>Chelydra serpentina serpentina</i>	X	X	X	X	X	X
western painted turtle	<i>Chrysemys picta bellii</i>	X	X	X	X	X	X
wood turtle	<i>Clemmys insculpta</i>	X	-	X	X	-	-
Blanding's turtle	<i>Emydoidea blandingii</i>	X	-	X	-	-	-
common map turtle	<i>Graptemys geographica</i>	X	X	X	X	-	-
false map turtle	<i>Graptemys pseudogeographica</i>	X	X	X	X	-	-
midland smooth softshell turtle	<i>Apalone mutica mutica</i>	X	-	X	-	-	-
spiny softshell turtle	<i>Apalone spinifera</i>	X	X	X	X	-	-
five-lined skink	<i>Eumeces fasciatus</i>	X	-	X	X	-	-
northern prairie skink	<i>Eumeces septentrionalis septentrionalis</i>	X	X	X	X	-	-
prairie racerunner	<i>Cnemidophorus sexlineatus viridis</i>	X	-	X	-	-	-
northern ring-necked snake	<i>Diadophis punctatus edwardsii</i>	X	-	X	-	X	-
western foxsnake	<i>Elaphe vulpina</i>	X	X	X	-	-	-
western hog-nosed snake	<i>Heterodon nasicus</i>	X	-	-	-	-	-
eastern hog-nosed snake	<i>Heterodon platirhinos</i>	X	-	X	X	-	-
eastern milksnake	<i>Lampropeltis triangulum triangulum</i>	X	-	X	-	-	-
northern watersnake	<i>Nerodia sipedon sipedon</i>	X	X	X	X	-	-
smooth greensnake	<i>Opheodrys vernalis</i>	X	-	X	X	-	-
bullsnake	<i>Pituophis melanoleucus sayi</i>	X	-	X	-	-	-
Texas brownsnake	<i>Storeria dekayi texana</i>	X	-	X	X	--	-
northern red-bellied snake	<i>Storeria occipitomaculata occipitomaculata</i>	X	X	X	X	X	X
plains gartersnake	<i>Thamnophis radix</i>	X	-	X	-	-	-
eastern gartersnake	<i>Thamnophis sirtalis sirtalis</i>	X	X	X	X	X	X
red-sided gartersnake	<i>Thamnophis sirtalis parietalis</i>	-	X	-	-	X	X
eastern yellow-bellied racer	<i>Coluber constrictor flaviventris</i>	X	-	X	-	-	-
eastern massasauga	<i>Sistrurus catenatus catenatus</i>	X	-	-	-	-	-
timber rattlesnake	<i>Crotalus horridus</i>	X	-	X	-	-	-

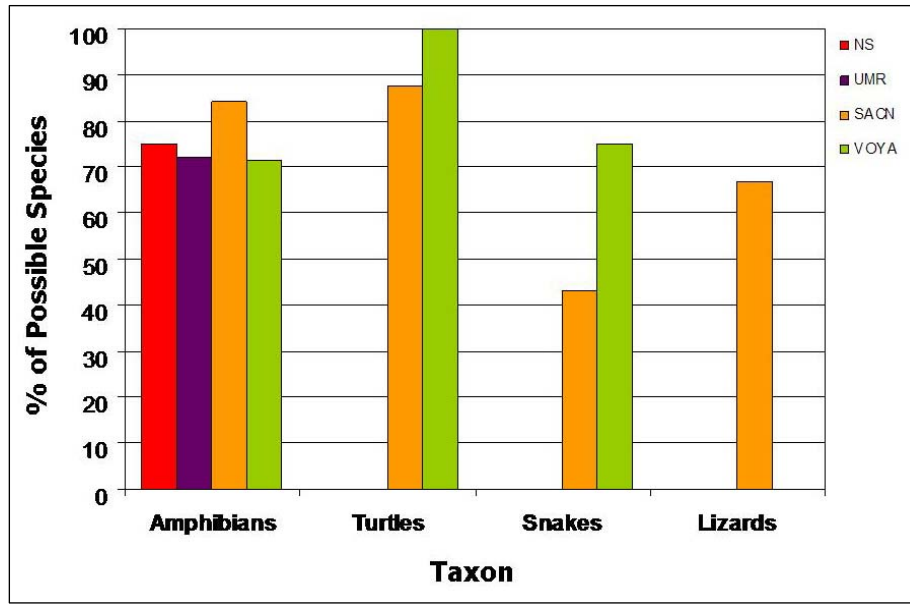


Figure 11. Percentage of possible species we actually observed across management units from 2002-2005.

Occupancy Estimates: We observed individual species in different proportions of the total number of breeding sites sampled in management units across years (Table 3). The total number of sites for each species in each management unit in Table 3 has not been adjusted to reflect the likely suitability of each breeding site for each species. In contrast, we did adjust the total number of sites we used in the occupancy analysis to reflect the potential breeding sites likely (according to our best predictions) available to each species in each management unit (see Methods). For these analyses, the number of potential breeding sites ranged from 59 (mink frogs-*R. septentrionalis*) to 197 (eastern American toad-*Bufo americanus*) across management units and the number of repeat surveys of these sites ranged from 54 (eastern gray treefrogs-*Hyla versicolor* and wood frogs-*Rana sylvatica*) to 62 (northern spring peepers-*Pseudacris crucifer* and green frogs-*R. clamitans*) across management units (Table 4; Data Supplement-Table 22).

Table 3. Proportions of sites where amphibian species were observed out of the total number of sites surveyed (daytime) within the Neal Smith National Wildlife Refuge (NS), 2004-2005, and the Upper Mississippi National Wildlife and Fish Refuge (UMR), St. Croix National Scenic Riverway (SACN), and Voyageurs National Park (VOYA) from 2002-2005. Dashes (-) indicate management units that are outside that species' range.

Management Unit	Blanchard's cricket frog	Chorus frog ¹	Bullfrog	Cope's gray tree-frog	Eastern gray tree-frog	Eastern American toad	Green frog	Mink frog	Northern Leopard frog	Plains leopard frog	Northern spring peeper	Pickrel frog	Wood frog	Central newt	Blue-spotted salamander	Spotted salamander	Eastern tiger salamander
NS	11/17	16/17	6/17	3/17	6/17	7/17	0/17	-	9/17	1/17	0/17	-	-	-	-	-	3/17
UMR	13/73	6/73	44/73	15/73	34/73	14/73	49/73	-	58/73	-	13/73	0/73	3/73	1/73	4/73	0/73	0/73
SACN	-	25/64	1/64	17/64	32/64	34/64	57/64	16/64	40/64	-	29/64	0/64	46/64	1/64	18/64	3/64	0/64
VOYA	-	9/57	0/57	0/57	20/57	21/57	23/57	15/57	33/57	-	31/57	-	52/57	8/57	17/57	-	-

¹The western chorus frog inhabited the NS and the UMR, and the boreal chorus frog inhabited VOYA. The two species overlapped theoretically in the SACN, where we did not attempt to differentiate between them.

Table 4. Occupancy analysis results by species from all ARMI management units including the Neal Smith National Wildlife Refuge from 2004-2005 and the Upper Mississippi National Fish and Wildlife Refuge, the St. Croix National Scenic Riverway and Voyageurs National Park from 2002-2005. Covariates used in models included date (DATE), time (TIME), observer (OBS1), management unit (MAN), surface area of the site (AREA), and wetland type (Habitat).

Presence ¹ model by species	sites	N ²	AIC	$\hat{\psi}$ ³	$\hat{\psi}$ (SE)	$\hat{\gamma}$ ⁴	$\hat{\gamma}$ (SE)	$\hat{\varepsilon}$ ⁵	$\hat{\varepsilon}$ (SE)	$\hat{\rho}$ ⁶ RANGE	$\hat{\rho}$ (SE) RANGE
chorus frog (ψ (), γ (), ε (), ρ (OBS MAN HABITAT)) [21]	197	61	1379.27	0.29	0.78	0.1	0.26	0.09	0.13	0.0-0.85	0.0-3.58
eastern American toad (ψ (), γ (), ε (), ρ (DATE MAN)) [8]	197	56	1426.16	0.47	0.9	0.18	0.77	0.7	0.73	0.0-0.34	0.0-1.37
eastern gray treefrog (ψ (), γ (), ε (), ρ (DATE HABITAT)) [17]	196	54	1614.6	0.6	0.79	0.21	0.63	0.48	0.99	0.0-0.61	0.0-1.60
northern green frog (ψ (), γ (), ε (), ρ (DATE OBS MAN HABITAT)) [17]	111	62	3881.47	0.79	0.05	0.44	0.07	0.21	0.04	0.18-1.0	0.0-0.13
mink frog (ψ (), γ (), ε (), ρ (DATE TIME OBS AREA HABITAT)) [16]	59	56	1014.57	0.48	0.09	0.06	0.04	0.07	0.05	0.0-0.67	0.00-0.14
northern leopard frog (ψ (), γ (), ε (), ρ (DATE OBS MAN AREA HABITAT)) [22]	128	60	3121.5	0.7	0.06	0.29	0.06	0.22	0.04	0.00 -0.76	0.00-0.17
northern spring peeper (ψ (), γ (), ε (), ρ (DATE TIME OBS MAN AREA)) [10]	180	62	36677.37	0.55	0.89	0.12	0.32	0.47	0.94	0.0-0.42	0.0-157.80
wood frog (ψ (), γ (), ε (), ρ (DATE TIME MAN HABITAT)) [18]	134	54	2677.22	0.61	0.06	0.51	0.07	0.2	0.04	0.0-0.70	0.0-0.08

¹ PRESENCE (<http://www.mbr-pwrc.usgs.gov/software.html>); Note: empty parentheses indicate no covariates applied to that parameter

² Number of Sampling occasions

³ Occupancy estimate

⁴ Colonization estimate

⁵ Extinction estimate

⁶ Detection probability

The sampling-specific covariates that were influential in the best-fitted occupancy models most often were time, observer, and date. Similarly, the site-specific covariates were management unit and habitat. Occupancy estimates for potential breeding sites within our relevant areas of inference ranged from 0.29 (chorus frog, *P. triseriata/maculata*) to 0.79 (green frog) with standard errors for occupancy from 0.05 (green frog) to 0.9 (eastern American toad). Ranges for the probabilities of detection ranged from 0-0.34 (eastern American toad) to 0.18-1.0 (green frog) with standard error ranges for detection probabilities from 0-0.13 (green frog) to 0-157.8 (northern spring peeper) (Table 4; Data Supplement-Table 22).

Metamorph Abnormality Surveys

We sampled very few metamorphs in the NS in 2004 and 2005 and observed no abnormalities. Percentage of abnormal metamorphs across all species for VOYA, the UMR, and the SACN from 2002-2005 ranged from 0.5 to 4.5, and means for each management unit across years were 1.5, 2.4, and 1.4, respectively (Table 5; Data Supplement-Figure 7; Data Supplement-Table 23).

Table 5. Number of metamorph abnormality surveys and percent abnormal metamorphs observed across all species for the Neal Smith National Wildlife Refuge (NS), 2004-2005, and the Upper Mississippi National Wildlife and Fish Refuge (UMR), the St. Croix National Scenic Riverway (SACN), and Voyageurs National Park (VOYA) from 2002-2005¹.

Management Unit	# of Surveys	# Caught	Abnormal	% Abnormal
NS 2004	16	18	0	0
NS 2005	4	23	0	0
NS Total	20	41	0	0
UMR 2002	53	413	2	0.48
UMR 2003	78	671	15	2.24
UMR 2004	48	367	5	1.36
UMR 2005	35	235	4	1.70
UMR Total	214	1686	26	1.54
SACN 2002	44	534	24	4.49
SACN 2003	94	593	13	2.19
SACN 2004	70	534	8	1.50
SACN 2005	38	303	3	0.99
SACN Total	246	1964	48	2.44
VOYA 2002	35	552	6	1.09
VOYA 2003	70	667	5	0.75
VOYA 2004	76	742	15	2.02
VOYA 2005	64	283	6	2.12
VOYA Total	245	2244	32	1.43

¹ We conducted surveys when we estimated species were metamorphosing. Numbers caught reflect timing of surveys with actual timing of metamorphosis and environmental conditions, such as wetland drying, rather than the actual number of metamorphs across species.

Collections for General Health Screening

From 2002-2005, we submitted 182 individual amphibians of several species, ranging from embryos to adults, to David Green at the National Wildlife Health Center for examination for abnormalities, parasites, and symptoms of disease (Data Supplement-Tables 24-26). Seventy animals were from VOYA, 59 were from the SACN, and 53 were from the UMR. Various parasites were present across species and management units, including the trematode, *R. ondatrae*, known to cause limb deformities. The potentially deadly disease, chytridiomycosis, was observed in two recently metamorphosed green frogs and six recently metamorphosed mink frogs from three sites in VOYA.

Sampling for *Batrachochytrium dendrobatidis*

Detection and relative amounts of Bd on metamorphs varied with management unit (Figure 12), site, and species in both years (Table 6; For more details, see Data Supplement-Tables 27-30).

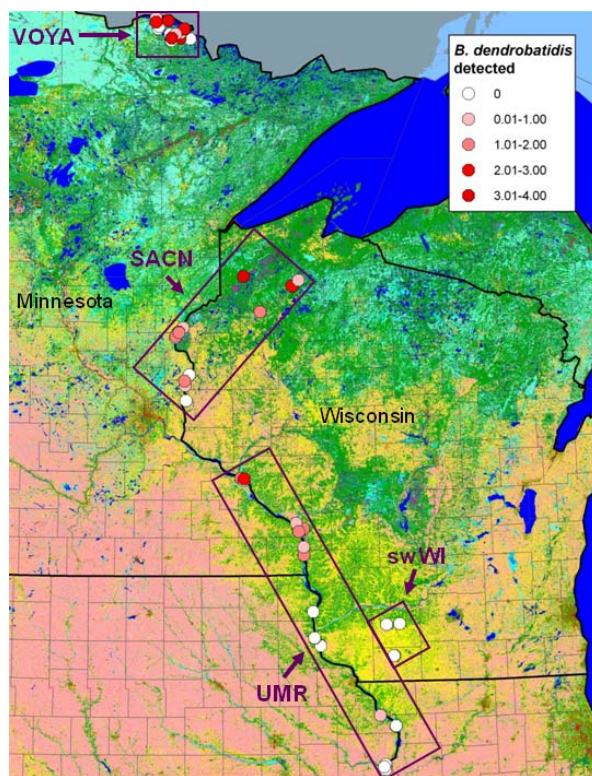


Figure 12. Relative quantities of *Batrachochytrium dendrobatidis* detected on recent amphibian metamorphs from sites in the UMR, the SACN, VOYA, and four breeding sites in southwestern Wisconsin during 2006 and 2007. Note that sampling sites often overlap each other within management units. See Table 6 for more details.

PCR analyses of our 2006 samples detected Bd on northern leopard frogs from two of the eight sites where we sampled them in the UMR. Bd was not detected on northern leopard frogs from

one site each in the SACN and VOYA, but was detected on green frogs from eight of eight sites in the SACN and three of three sites in VOYA, and on mink frogs from six of six sites in VOYA. Bd was not detected on Blanchard's cricket frogs at any of the four sites on state lands in Wisconsin (Table 6; Data Supplement-Tables 27-30).

For our 2007 samples, Bd was detected on northern leopard frogs from two of 15 sites in the UMR and from one of six sites in VOYA, but not from either of two sites in the SACN. It also was detected on green frogs from eight of ten sites in the UMR, six of nine sites in the SACN, and two of three sites in VOYA; on mink frogs from the one site we sampled them in the SACN and five of six sites in VOYA; on wood frogs from two of five sites sampled in the SACN and four of four sites in VOYA. Bd was not detected on boreal/western chorus frogs from two sites sampled in the SACN, on eastern gray treefrogs from one site in the SACN and four sites in VOYA, or on eastern American toads from one site in the UMR (Table 6; Data Supplement-Tables 27-30).

In 2006 and 2007, Bd was detected more often and in relatively larger quantities on more aquatic species, such as green frogs and mink frogs. This was most evident in the results from analyses of more than one species from the same site, such as leopard frogs and green frogs in the UMR, or green and/or mink frogs and leopard frogs in VOYA (Table 6; Data Supplement-Tables 27-30). Wood frogs, a relatively non-aquatic species, were an exception to this pattern.

Table 6. Relative quantities of *Batrochytrium dendrobatidis* (Bd) detected in extracts from 10 pooled swabs¹ of recent metamorphs by species, year, area, and site. Acb = *Acris crepitans blanchardi*, Rcl = *Rana clamitans*, Rp = *R. pipiens*, Rse = *R. septentrionalis*, Ba = *Bufo americanus*, Hv = *Hyla versicolor*, Pt = *Pseudacris triseriata*, Rca = *R. catesbeiana*, and Rsy = *R. sylvatica*. 0 = not detected, 1 = weak positive signal, 2 = positive signal, 3 = strong positive signal, and 4 = very strong positive signal, as reported by Pisces Analytical LLC.

Area ² /Site	Species/Year											
	Acb	Rcl		Rp		Rse		Ba	Hv	Pt	Rca	Rsy
	2006	2006	2007	2006	2007	2006	2007	2007	2007	2007	2007	2007
UMR												
P4DA1	-	-	0,0 ⁽³⁾	0,0	0,0,0	-	-	-	-	-	-	-
P4DA2	-	-	0,3,3	-	-	-	-	-	-	-	-	-
P4DC1	-	-	0,0,0	-	0,0,0	-	-	-	-	-	-	-
P4DD1	-	-	3,4,4	0,3	0,0,4	-	-	-	-	-	-	-
P7DAI1	-	-	2,3,3	-	-	-	-	0,0,0	-	-	-	-
P7DE3	-	-	0,2,3	-	0,0,0	-	-	-	-	-	-	-
P7DH1	-	-	0,3,4	-	0,0,0	-	-	-	-	-	-	-
P8DB1	-	-	3,3,4	0,0	0,0,0	-	-	-	-	-	-	-
P8DG1	-	-	-	0,0	0,0,3	-	-	-	-	-	-	-
P10DA3	-	-	0,0,2	-	-	-	-	-	-	-	-	-
P10DA4	-	-	-	-	0,0,0	-	-	-	-	-	-	-
P10DB3	-	-	4	-	0,0,0	-	-	-	-	-	-	-
P10DB4	-	-	-	0,0	-	-	-	-	-	-	-	-

Table 6. Relative quantities of *Batrochytrium dendrobatidis* (Bd) detected in extracts from 10 pooled swabs¹ of recent metamorphs by species, year, area, and site (continued).

Area ² /Site	Species/Year											
	Acb	Rcl		Rp		Rse		Ba	Hv	Pt	Rca	Rsy
	2006	2006	2007	2006	2007	2006	2007	2007	2007	2007	2007	2007
<i>UMR</i>												
P10DD1	-	-	-	0,0	0,0,0	-	-	-	-	-	-	-
P13DA1	-	-	-		0,0,0	-	-	-	-	-	-	-
P13DA4	-	-	-	0,0	-	-	-	-	-	-	-	-
P13DA6	-	-	-	-	-	-	-	-	-	-	0 ⁽⁵⁾	-
P13DB3	-	-	-	0,2	0,0,0	-	-	-	-	-	-	-
P14DA1	-	-	-	-	0,0,0	-	-	-	-	-	-	-
P14DB2	-	-	-	-	0,0,0	-	-	-	-	-	-	-
P14DC1	-	-	-	-	0,0,0	-	-	-	-	-	-	-
<i>SACN</i>												
SC1DA1	-	3,4	0,0,0	-	0,0,0	-	-	-	-	-	-	-
SC1DE1	-	-	0,0,0	-	-	-	-	-	-	-	-	-
SC2DB1	-	-	0,0,0	-	-	-	-	-	-	-	-	-
SC2DD2	-	3,4	0,0,3	-	-	-	-	-	-	-	-	0,0,2
SC4DA3	-	-	0,3,3	-	-	-	-	-	-	-	-	0,0,0
SC4DB1	-	-	0,3,3	-	-	-	-	-	-	-	-	-
SC4DB2	-	2,2	-	-	-	-	-	-	-	-	-	-
SC4DB9	-	-	-	-	-	-	-	-	-	0,0,0	-	0,0,0
SC4DBI2	-	-	-	-	-	-	-	-	-	0,0,0	-	0,0,0
SC4DC1	-	2,4	-	-	-	-	-	-	-	-	-	0,0,3
SC4DCI2	-	-	-	-	-	-	-	-	0,0,0	-	-	-
SC8DB1	-	4,4	-	-	-	-	-	-	-	-	-	-
SC10DC1	-	1,3	-	-	-	-	-	-	-	-	-	-
SC12DB1	-	3,4	0,3,3	0,0	0,0,0	-	-	-	-	-	-	-
SC12DC1	-	-	2,3,3	-	-	-	-	-	-	-	-	-
SC12DE1	-	0,2	0,0,2	-	-	-	0 ⁽⁵⁾ ,2	-	-	-	-	-
<i>VOYA</i>												
V1DB1	-	-	-	-	-	-	-	-	0,0,0	-	-	-
V2DF1	-	4,4	3	-	-	4,4	3,4,4	-	-	-	-	-
V3DAI1	-	-	-	-	0,0,0	-	0,0,0	-	0 ⁽¹⁾	-	-	0,0,3
V3DC1	-	-	-	-	-	0,4	0,0,2	-	-	-	-	-
V4DC1	-	0,3	2,2,3	-	0 ⁽⁷⁾	3,4	3,3,4	-	-	-	-	-
V4DC2	-	-	-	-	-	-	-	-	-	-	-	2,2,3
V5DA1	-	-	-	-	-	4,4	-	-	-	-	-	-
V5DA2	-	-	-	-	-		2,4,4	-	-	-	-	-
V7DE1	-	-	-	0,0	0,0,0	-	-	-	0,0,0	-	-	0,0,3
V8DB1	-	-	-	-	-	0 ⁽¹⁾	-	-	-	-	-	-
V8DC1	-	-	-	-	0,0,0	-	-	-	-	-	-	0,0,4
V8DC3	-	-	-	-	-	4,4	-	-	-	-	-	-
V9DBI1	-	-	-	-	-	-	-	-	0	-	-	-
V10DC1	-	-	-	-	-	-	-	-	0,0,0	-	-	-
V11DA1	-	0,2	0,0 ⁽¹⁾	-	0 ⁽⁹⁾	-	-	-	-	-	-	-

Table 6. Relative quantities of *Batrochytrium dendrobatidis* (Bd) detected in extracts from 10 pooled swabs¹ of recent metamorphs by species, year, area, and site (continued).

Area ² /Site	Species/Year											
	<i>Acb</i>	<i>Rcl</i>		<i>Rp</i>		<i>Rse</i>		<i>Ba</i>	<i>Hv</i>	<i>Pt</i>	<i>Rca</i>	<i>Rsy</i>
	2006	2006	2007	2006	2007	2006	2007	2007	2007	2007	2007	2007
VOYA												
V11DB4	-	3 ⁽¹⁾	-	-	0,2,4	4,4	4	-	-	-	-	-
swWI												
CP	0,0	-	-	-	-	-	-	-	-	-	-	-
MP	0,0	-	-	-	-	-	-	-	-	-	-	-
RG	0,0	-	-	-	-	-	-	-	-	-	-	-
TV	0,0	-	-	-	-	-	-	-	-	-	-	-

¹Numbers in parenthetical superscripts indicate number of pooled swabs used if <10.

²UMR = Upper Mississippi River National Wildlife and Fish Refuge; SACN = St. Croix National Scenic Riverway; VOYA = Voyageurs National Park; swWI = southwestern Wisconsin.

Nighttime Surveys

We intended our observations of calling amphibians during nighttime call surveys to complement results from daytime sampling efforts at nearby breeding sites (Data Supplement-Table 13). Nighttime call surveys confirmed the presence of species observed during daytime call and visual-encounter surveys at all locations (Tables 3 and 7). In addition, we observed pickerel frogs, *R. palustris*, in the UMR during nighttime surveys (Table 7; Data Supplement-Table 19) where we had not detected them during the day. While we did not detect any other species only during nighttime surveys, we typically detected northern spring peepers, eastern gray and Cope's gray treefrogs (*H. chrysoscelis*), and Blanchard's cricket frogs more frequently during nighttime surveys (Tables 3 and 7).

Targeted Searches and Cover Objects—Amphibians and Reptiles

Voyageurs National Park and the St. Croix National Scenic Riverway

Our searches under natural cover objects in cells and opportunistic observations resulted in sightings of four-toed salamanders (*Hemidactylum scutatum*) and eastern redback salamanders (*Plethodon cinereus*) in the SACN that we did not observe there otherwise. Given we later observed a Blanding's turtle (*Emydoidea blandingii*) in the SACN in 2007, the only turtle species we did not observe in this park was the midland smooth softshell (*Apalone mutica mutica*). We observed all potential turtle species in VOYA (Data Supplement-Tables 31 and 32). Similarly, we observed 43% of the possible snakes and 67% of the possible lizards in the SACN and 75% of the possible snakes in VOYA (Table 2). We did not expect lizards to be present in VOYA.

We did not observe any amphibians or reptiles under any of the 55 cover boards we had deployed in the SACN. We observed one boreal chorus frog under one of the 36 cover boards in VOYA and one wood frog under another cover board in the park.

Table 7. Proportions of sites where amphibian species were observed out of the total number of sites surveyed (nighttime) within the Neal Smith National Wildlife Refuge (NS), 2004-2005, and the Upper Mississippi National Wildlife and Fish Refuge (UMR), the St. Croix National Scenic Riverway (SACN), and Voyageurs National Park (VOYA) from 2002-2005. Dashes (-) indicate management units that are outside that species' range.

Management Unit	Blanchard's cricket frog	Chorus frog ²	Bullfrog	Cope's gray tree- frog	Eastern gray tree-frog	Eastern American toad	Green frog	Mink frog	Northern leopard frog	Northern spring peeper	Pickrel frog	Wood frog
NS	7/7	7/7	7/7	7/7	7/7	7/7	0/7	-	7/7	0/7	-	-
UMR	3/8	4/8	5/8	6/8	6/8	6/8	8/8	-	6/8	5/8	1/8	3/8
UMR 20051	10/34	11/34	15/34	4/34	28/34	22/34	25/34	-	13/34	15/34	4/34	0/34
SACN	-	6/10	1/10	4/10	8/10	8/10	7/10	2/10	5/10	10/10	0/10	7/10
SACN 20051	-	19/36	3/36	4/36	35/36	28/36	25/36	5/36	9/36	21/36	0/36	8/36
VOYA	-	1/4	0/4	0/4	4/4	3/4	4/4	4/4	4/4	4/4	-	4/4

¹For UMR 2005 and SACN 2005, each random night survey node was treated as an individual site.

²The western chorus frog inhabited the NS and the UMR, and the boreal chorus frog inhabited VOYA. The two species overlapped theoretically in the SACN, where we did not attempt to differentiate between them.

Mississippi National River and Recreation Area

We observed 31% of the amphibian species for which we conducted targeted searches at MISS (Table 8). We did not observe either of the two species of snakes we targeted in the MISS, but we did observe other snake species we had not targeted, including the red-sided garter snake (*Thamnophis sirtalis parietalis*), which the NPS did not have on their list of species likely present in the MISS. We observed the northern prairie skink (*Eumeces septentrionalis septentrionalis*), which appeared quite common, of the three lizard species possible. We observed five of the eight turtles potentially present at MISS (Table 8) and all species we had targeted except the midland softshell turtle (Table 8).

Table 8. Reptiles and amphibians targeted and observed at MISS.

Common Name	Scientific Name	ARMI-MR targeted	ARMI-MR confirmed
blue-spotted salamander	<i>Ambystoma laterale</i>	X	-
spotted salamander	<i>Ambystoma maculatum</i>	X	-
eastern tiger salamander	<i>Ambystoma tigrinum tigrinum</i>	X	X
midland smooth softshell	<i>Apalone mutica mutica</i>	X	-
spiny softshell	<i>Apalone spinifera</i>	X	X
prairie racerunner	<i>Cnemidophorus sexlineatus viridis</i>	X	-
northern ring-necked snake	<i>Diadophis punctatus edwardsii</i>	X	-
five-lined skink	<i>Eumeces fasciatus</i>	X	-
northern prairie skink	<i>Eumeces septentrionalis septentrionalis</i>	X	X
common map turtle	<i>Graptemys geographica</i>	X	X
false map turtle	<i>Graptemys pseudogeographica</i>	X	X
four-toed salamander	<i>Hemidactylum scutatum</i>	X	-
Cope's gray treefrog	<i>Hyla chrysoscelis</i>	X	-
eastern gray treefrog	<i>Hyla versicolor</i>	X	X
common mudpuppy	<i>Necturus maculosus maculosus</i>	X	-
central newt	<i>Notophthalmus viridescens louisianensis</i>	X	-
eastern red-backed salamander	<i>Plethodon cinereus</i>	X	-
northern spring peeper	<i>Pseudacris crucifer crucifer</i>	X	-
boreal chorus frog ¹	<i>Pseudacris maculata</i>	X	X
western chorus frog ¹	<i>Pseudacris triseriata</i>	X	X
American bullfrog	<i>Rana catesbeiana</i>	X	X
pickerel frog	<i>Rana palustris</i>	X	-
mink frog	<i>Rana septentrionalis</i>	X	-
wood frog	<i>Rana sylvatica</i>	X	-
Texas brownsnake	<i>Storeria dekayi texana</i>	X	-

¹We did not differentiate between the boreal chorus frog and the western chorus frog.

Discussion

To our knowledge, our results provide the first regional view of the statuses of amphibian populations and their exposures to two potential causes of decline on DOI lands in the Midwest. Furthermore, these results are based upon extensive daytime and nighttime surveys conducted according to the same protocols across management units. While some extrapolations regarding current species presence and distribution in these DOI areas might be possible based upon previous summaries for the Midwest (e.g., Lannoo 1998; Lannoo 2005) or, perhaps more likely, recent call-survey data from individual states (Iowa DNR 2007; Minnesota DNR 2007; Wisconsin DNR 2007), these sources largely do not provide specific information on breeding sites and stressors, or caudates in the case of call-survey data, among other shortcomings. This complementary information provides resource managers in the NS, UMR, SACN, and VOYA with key baseline information specific to their areas that also can be compared across these management units in a regional context.

These results overall show that many of the expected amphibian and reptile species were present to varying extents in these management units. They also show that some amphibian breeding populations were exposed to concentrations of triazine herbicides similar to those shown to induce endocrine disrupting effects in amphibians in laboratory tests (Hayes et al. 2002b). These exposures were greater in the NS and UMR, which are more linked geographically to extensive row crop agriculture. Alternatively, the potentially lethal chytrid fungus, *B. dendrobatidis*, was present at breeding sites across the UMR, the SACN, and VOYA. The data we report here do not allow us to speak to trends in the presence of amphibian species in the NS, UMR, SACN, and VOYA over time. They do provide a comprehensive regional snapshot of species presence and exposure to these potentially important stressors.

Daytime Amphibian Surveys–Species Observed

We observed most of the amphibian species we expected to be present in VOYA, the SACN, the UMR, and the NS based upon predictions and our sampling methods (Table 1; Figure 13; Data Supplement-Figure 8; Data Supplement-Tables 12, 14-21). These species occupied wetlands that varied substantially in their physical and chemical characteristics.

Individual species varied from relatively common to uncommon at the breeding sites we sampled. This, to some extent, reflects relative presence. However, based upon the results of our occupancy analysis (Table 4; Data Supplement-Table 22), our confidence in our ability to detect individual species varied greatly. Probabilities of detection were low with high variance for some species, such as spring peepers, while we were much more confident we detected other species, such as green frogs, when they were present (Table 4; Data Supplement-Table 22). This complementary information regarding the probability of detection and associated variance is one of the strengths of conducting the occupancy analysis in that it qualifies the estimated occupancy in terms of confidence in that estimation based upon one's ability to detect the species being sampled (MacKenzie and Kendall 2002; MacKenzie et al. 2002).

Based upon the small number of reports of using occupancy analyses on amphibian data similar to ours, but for terrestrial salamanders (Bailey et al. 2004) and southeastern anurans (Smith et al. 2006), our estimates of occupancies and detection probabilities were similar to previous reports

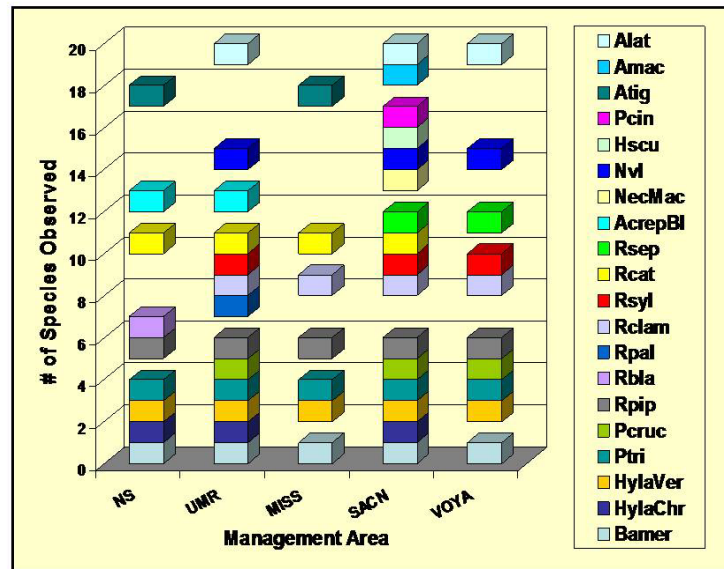


Figure 13. Number and species of amphibians we observed across management areas. Alat = *Ambystoma laterale*, Amac = *A. maculatum*, Atig = *A. tigrinum*, Pcin = *Plethodon cinereus*, Hscu = *Hemidactylum scutatum*, Nvl = *Notophthalmus viridescens louisianensis*, NecMac = *Necturus maculosus*, AcrepBl = *Acris crepitans blanchardi*, Rsep = *Rana septentrionalis*, Rcat = *R. catesbeiana*, Rsyl = *R. sylvatica*, Rclam = *R. clamitans*, Rpal = *R. palustris*, Rbla = *R. blairi*, Rpip = *R. pipiens*, Pcruc = *Pseudacris crucifer*, Ptri = *P. triseriata*, HylaVer = *Hyla versicolor*, HylaChr = *H. chrysoscelis*, Bamer = *Bufo americanus*.

for some species and dissimilar for others. The variances on our detection probabilities appear to be greater as a whole. Our detection probabilities *per se* were produced as ranges for the multi-year occupancy estimates and are difficult to compare directly to the results of single-year occupancy estimates produced in these previous studies.

The potential reasons for differences in values among such studies are numerous (Bailey et al. 2004; Smith et al. 2006). They range from differences in the distributions and abundances of species and how cryptic individual species are to the effectiveness of methods employed and the number of sampling events. Our occupancy analyses were based upon results from daytime surveys, during which amphibians typically do not call as frequently or in the same numbers compared with later in the day and animals small in size and/or low in number are difficult to detect visually. While we detected calling spring peepers easily at night, they were considerably more difficult to detect during the day due to sporadic calling, embryos that are difficult to detect, small size across life stages and age classes, and the ability of post-metamorph age classes to remain hidden. Green frogs, on the other hand, produce much larger egg masses, and all age classes are easier to catch and see. Other species fall somewhere in this spectrum and our

probabilities of detecting them, and in turn our estimates of occupancy, reflected these characteristics to some extent.

Nighttime Amphibian Surveys–Species Observed

Many of the attributes that can make small camouflaged anurans challenging to detect during daytime sampling are negated during nighttime surveys when males of species that call are easily heard. Although we did not calculate occupancy estimates and detection probabilities for call data from nighttime surveys, we observed Blanchard’s cricket frogs, chorus frogs, treefrogs, and northern spring peepers at a relatively high proportion of sites (Table 6; Data Supplement-Tables 18-21). This was true for eastern American toads as well, another species for which the occupancy estimate and detection probability were low in our analyses. This speaks to the potential effectiveness of conducting daytime and nighttime surveys for calling anurans when resources permit.

Daytime and Nighttime Amphibian Surveys–Species Not Observed

The amphibian species we did not observe in VOYA, the SACN, the UMR, and the NS were ones for which we did not survey, likely were not present, or likely were uncommon or rare (Conant and Collins 1998; ARMI Atlas 2007; Wisconsin Herp Atlas Project 2007). We found few of the species we expected in the MISS, but we greatly restricted our efforts there and our MISS results are not easily compared to those from other management units.

Voyageurs National Park

We did not observe eastern redback salamanders in VOYA despite extensive searching under natural and artificial cover. Nor did we observe Canadian toads (*Bufo hemiophrys*) despite physically examining many toads of all age classes in the park. We did not sample for common mudpuppies in VOYA. Theoretically, these three species could be present in the park due to the fact that the edges of their described distributions are reasonably near the park to varying degrees (ARMI Atlas 2007). Given the extent to which we sampled for eastern redback salamanders and Canadian toads, these species are uncommon at best if they are present in VOYA.

St. Croix National Scenic Riverway

We did not observe the eastern tiger salamander or the pickerel frog in the SACN. These species are likely to occur in the SACN based upon reliable historical records (ARMI Atlas 2007; Wisconsin Herp Atlas Project 2007) and the apparently known occurrence of eastern tiger salamanders at Interstate State Park abutting the SACN near Taylor Falls, Minnesota. However, they are not common in the SACN based upon our extensive daytime and nighttime sampling for amphibians. Based upon NPS historical records, Blanchard’s cricket frogs also were observed at two locations in the SACN. While the occurrence of this species in the SACN is possible, we did not observe it despite our searches in the vicinities of past reported sightings and our extensive call surveys in the park.

The SACN is an area of likely overlap in the distributions of the western chorus frog and the boreal chorus frog (ARMI Atlas 2007). To our knowledge, these species are not reliably separable based upon calls or gross physical appearance. Thus, we treated chorus frogs we observed in the SACN as being of either species or possibly hybrids between the two.

Mississippi National River and Recreation Area

We had very limited resources to conduct targeted searches for amphibians in the MISS. Of the set of species the NPS asked us to potentially target in the MISS, we targeted a subset (Tables 1 and 8) based upon the resources available and likely habitat availability. Of that subset, we could not further distinguish the chorus frogs we observed as western or boreal and, although we did not observe common mudpuppies, biologists from Fort Snelling State Park told us of confirmed reports of fishermen catching mudpuppies in the MISS. Of our remaining targeted species, we did not observe blue-spotted (*A. laterale*), spotted (*A. maculatum*), and eastern redback salamanders, central newts (*Notophthalmus viridescens louisianensis*), Cope's gray treefrogs, northern spring peepers, wood frogs, and pickerel frogs.

We did not observe blue-spotted and spotted salamanders and central newts, despite daytime sampling of several likely breeding sites (J. Moriarity, personal communication; personal observations) and searches of cover objects, but we did not census all potential habitat. Similarly, we did not observe Cope's gray treefrogs, northern spring peepers, wood frogs, and pickerel frogs during daytime and nighttime surveys of a limited number of breeding sites. Northern spring peepers and wood frogs in particular should have been relatively easy to detect via nighttime call surveys. All of these species could still occur in the MISS, despite some of their described distributions being somewhat removed from or on the edge of the area (ARMI Atlas 2007) and its largely urban, suburban, and agricultural landscape, but we suspect they are not common. More sampling is needed to have higher confidence in the potential presence of the species we did not observe or attempt to sample.

Upper Mississippi River National Wildlife and Fish Refuge

We observed all anurans expected to be present in the UMR (Data Supplement-Table 12). We did not observe spotted salamanders, small-mouth salamanders (*A. texanum*), eastern tiger salamanders, common mudpuppies, and four-toed salamanders. To some extent this reflects the relative ease of detecting most anurans via call surveys. It might also reflect greater challenges for some salamander species to persist in the floodplain of the Mississippi River where intermittent flooding from the river's channels can affect the quality and quantity of breeding and upland habitats.

Common mudpuppies can live within the channels of the Mississippi River or in other wetland types in the floodplain. We know they are present in the UMR based upon many anecdotal accounts of them being caught by people while fishing. We did not sample for them, but likely would have observed them if we had. Small-mouth salamanders likely are present only in Pool 14, if at all. Although we did sample amphibian breeding sites in Pool 14, we did not observe this species.

Eastern tiger salamanders likely are present in select habitat in the UMR, but based upon our visual-encounter sampling they are not common. The presence of four-toed salamanders in the UMR is questionable given their distribution (ARMI Atlas 2007) and ecology (Lannoo 2005), but not impossible given the matrices of wetland and terrestrial habitats in the floodplain.

Neal Smith National Wildlife Refuge

We did not observe small-mouth salamanders at the NS, perhaps due to the limited combination of appropriate wooded upland habitat and aquatic breeding habitat available on this refuge. Given our relatively extensive sampling of wetlands suitable as breeding habitat for this species, it likely is not common at the NS.

We did not observe the green frog and the northern spring peeper at the NS. The described ranges of these species are near the NS (ARMI Atlas 2007), but the fact we did not hear them during repeated nighttime call surveys across years suggests they are uncommon at best. In addition, the USFWS had been eliminating former stock ponds on the refuge. These were the only wetlands at the NS with sufficiently permanent hydroperiods to support green frogs.

Regarding species we did observe at the NS, this refuge is located in an area of overlap between northern leopard frogs and plains leopard frogs. We observed both species and might have unknowingly observed hybrids of the two as well. This refuge also is located just south of the apparent decline zone of Blanchard's cricket frogs in Iowa. This species was common at the NS.

Exposures to Triazine Herbicides

Amphibians were exposed to triazine herbicides at breeding sites during the reproductive seasons of 2004 and 2005 in all management units we sampled, although considerably more often and in greater concentrations at breeding sites in the NS and UMR than at sites in the SACN and VOYA. This is not surprising, given that the NS and UMR in general are much closer to extensive row crops, especially corn (Figure 2; Data Supplement-Figure 6), where atrazine is applied voluminously each year (Data Supplement-Figure 2). Our ELISA measurements did not necessarily reflect pure concentrations of atrazine parent compound because the antibodies used in the ELISA also react with other triazines to varying degrees (Abraxis 2007). However, the results of our parallel LCMS analysis showed the atrazine parent compound and its breakdown products dominated the triazine compounds found in our samples, most likely due to runoff and atmospheric deposition of atrazine applied to corn in the region.

Triazine concentrations measured via the ELISA were greater than 0.1 ppb at a substantial number of breeding sites. Atrazine is known to function as an endocrine disruptor and this concentration of atrazine has been shown to cause abnormal gonads in native northern leopard frogs and non-native African clawed frogs in laboratory tests (Hayes et al. 2002b; 2003). Whether or not atrazine induces such effects in amphibians at these concentrations in the wild in the face of multiple interacting factors is not clear, but Hayes et al. (2003) reported similar gonadal abnormalities in leopard frogs collected from midwestern field sites where exposures to atrazine were greater than 0.2 ppb. Gonadal abnormalities among northern leopard frogs we collected from a small number of sites in VOYA, the SACN, and the UMR and analyzed by Dr. Hayes did not appear to be associated consistently with triazine concentrations at the sites we collected them (data not presented here), but this effort and the results were preliminary and the dynamics affecting such cause-and-effect relationships are complex (Hayes et al. 2003).

An example of how the variability in the wild could affect exposure to pesticides and any biological effects is evident from the results of our intensive sampling of sites in the UMR in 2004 and 2005 (Figure 10; Data Supplement-Tables 8-11). Triazine concentrations not only

varied among breeding sites on a given date, they fluctuated over time in association with the hydrograph of the Mississippi River. Changes in the hydrograph presumably were driven by precipitation events on the upland landscape and subsequent runoff into tributaries that empty into the floodplain. Triazine concentrations at breeding sites then likely were a function of when farmers applied atrazine to their fields, the timing and extent of precipitation events, and the distribution of water in the floodplain, especially if water from the channels of the Mississippi River flooded and inundated breeding sites in the floodplain that were slightly higher in elevation and often not linked to the channels by surface water.

The timing of breeding, hatching, and larval development for the amphibian species living in the floodplain in relation to these human, climatic, and hydrological factors would have dictated the extent of larval exposure to potential effects-inducing concentrations during the supposed narrow windows of larval development when endocrine disruption can occur (Hayes et al. 2003). Thus, relationships between climate, land use, the fate and transport of pesticides, exposure to toxic concentrations, exposure windows during development, and effects in amphibians and other organisms in nature are complex and variable and need to be accounted for in understanding the dynamics between the applications of pesticides and any effects on amphibians.

An added dimension to note is that mixtures of pesticides and pesticide interactions with other environmental factors can have synergistic effects on amphibians (e.g., Kiesecker 2002; Boone and James 2003; Relyea 2004, 2005; Davidson et al. 2007). Considerable need exists for more comprehensive, integrated studies of the effects of pesticides on wild populations *in situ* under variable natural conditions to better understand the predictive value of tests in the laboratory or mesocosms and effects on wild populations (e.g., Sparling et al. 2001; Davidson et al. 2002; Hayes et al. 2003; Davidson and Knapp 2007; Sparling and Fellers 2007).

Sampling for *Batrachochytrium dendrobatidis*

The results of our Bd surveys at breeding sites in VOYA, the SACN, and the UMR provide the first data on the presence of this potentially lethal pathogen in these areas and to a larger extent in the upper Midwest. Given the evidence that chytridiomycosis, the disease caused by Bd, has been associated with dramatic declines of amphibian populations around the world, including in protected areas such as national parks (Muths et al. 2003; Lips et al. 2006; Morgan et al. 2007; Skerratt et al. 2007), the presence of this organism at such a high percentage of the locations we sampled is cause for concern and further study.

However, it must be remembered that evidence also exists that suggests the presence of Bd does not always result in chytridiomycosis or die-offs (Corn 2007; Longcore et al. 2007; Pearl et al. 2007). Whether this is due to species and populations being differentially resistant or adapted to co-existing with Bd, die-offs having gone undetected, or conditions having not been suitable for this pathogen to wreak havoc on all populations is not clear (Longcore et al. 2007). David Green's examinations of a small number of 2002 and 2003 samples from the UMR, SACN, and VOYA showed chytridiomycosis present in two green frogs and six mink frogs from three breeding sites in VOYA (Data Supplement-Tables 24-26), which were located in sampling cells where we found Bd present in 2006 and 2007 (Table 6; Data Supplement-Table 29). We have not observed amphibian die-offs of consequence at the breeding sites where Bd was present or at any other sites we have sampled in these management units, but some could have escaped

detection. Coupled with the fact that we cannot assess trends in amphibian presence in these areas at this time and we do not know how changes in ecological conditions might increase the presence, transmissibility, infection rates, or virulence of Bd in these areas, further surveys and monitoring of the presence of population statuses relative to Bd, chytridiomycosis, and environmental conditions is prudent.

In general, we observed Bd more frequently on samples from green frogs and mink frogs compared to northern leopard frogs and other less aquatic species (Table 6; Data Supplement-Tables 27-30). This was true across management units. Wood frogs were somewhat of an exception to this pattern for reasons we do not understand currently. Our results of varying Bd presence on different species sampled at the same site are similar to other reports that Bd was detected more often on species that spend more time in the water (e.g., Pearl et al. 2007). Whether this is due to more prolonged exposure to the fungus for more aquatic species or combinations of factors is not clear.

Other Pathogens and Parasites

We collected the amphibian samples we submitted for health screening more opportunistically than according to a predetermined design for testing hypotheses. Despite limitations inherent in such a process, the results provide some useful information (Data Supplement-Tables 24-26).

Overall, these examinations showed the vast majority of all individuals were afflicted with parasites of one sort or another, despite outwardly healthy appearances. We do not know how often such parasite loads might result in reduced fitness among affected animals.

Our observations of chytridiomycosis in some animals, coupled with the results of our Bd sampling, suggest exposure to Bd resulted in expression of the disease at some sites. We did not observe other pathogens of note, such as Ranaviruses, and associated diseases in our samples.

Observations that the trematode, *R. ondatrae*, was present in some specimens and likely present in others showed the apparent principal causative agent of abnormalities (see Abnormalities section below) was present in some breeding sites.

Abnormalities

As described in the Background section, the discovery of amphibians with missing and extra limbs and other abnormalities in the Midwest and elsewhere served to further propel growing awareness of global amphibian declines in the 1990s. Since then a number of studies have shown that several abiotic and biotic factors can cause these deformities (Blaustein and Johnson 2003; Johnson et al. 2007); principal among these is infection by *R. ondatrae* (Blaustein and Johnson 2003; Johnson and Chase 2004; Johnson et al. 2007). As reflected in the extensive database of the North American Reporting Center for Amphibian Malformations (NARCAM 2007), gross physical abnormalities have been observed in many species in many locations in North America. Blaustein and Johnson (2003) summed observations of abnormalities in the United States as being from more than 60 species in 46 states, with 80% of the samples from some local populations showing abnormalities, frequencies that could cause amphibian population declines.

Results from the Midwest have shown some species at some individual sites had higher abnormality frequencies as well (Helgen et al. 2000), while animals at other sites, including sites on U.S. Fish and Wildlife Refuges, had consistently lower frequencies (Converse et al. 2000; Helgen et al. 2000). Close examination of the NARCAM database shows that a considerable majority of the reported abnormality frequencies are in the low single digits at best. Similarly, our survey results across VOYA, the SACN, the UMR, and the NS from 2002-2005 showed mean abnormality frequencies within and across management units and years of less than 5% (Table 5; Data Supplement-Figure 7). Regardless of whether frequencies less than 5% truly can be considered as natural background frequencies in healthy populations (Blaustein and Johnson 2003) or not, such frequencies do not appear to be sufficient to cause population declines. This assumes abnormalities did not kill large numbers of developing larvae or metamorphosing animals before we could observe them as metamorphs.

We should note that our abnormality sampling efforts were relatively opportunistic. We did not closely monitor larval development for individual species at a pre-selected set of sites in each management unit to be able to sample different species as they metamorphosed. We simply did not have the resources. As a result, we sampled mostly northern leopard frogs, green frogs, and mink frogs (e.g., see 2003-2005 data in Data Supplement-Table 23). In 2007, we began implementing more intensive sampling at a small number of breeding sites in relation to climate-change studies. We expect to monitor the development and metamorphosis of some additional species more closely at those sites in the future.

Ribeiroia ondatrae was present at different breeding sites across management units (Data Supplement-Tables 24-26). Given the proximity of breeding sites in the UMR and NS to potential agricultural runoff and Johnson et al.'s recent work (2007), closer examination of any relationships between nutrient runoff and atmospheric deposition, eutrophication, abnormalities, and survival at these sites would be worthwhile.

Reptile Surveys

We conducted limited surveys for reptiles in the SACN and VOYA, concentrating primarily on basking surveys for turtles, but also searching to a lesser extent under natural and artificial cover objects for other reptiles in the cells we sampled. We did not survey for reptiles in the NS or UMR.

We observed all of the turtle species likely to be present in VOYA. The only turtle species we did not observe in the SACN was the midland smooth soft-shelled turtle. This species could be present in the lower St. Croix River south of St. Croix Falls based upon its preferred habitat of large flowing water (Donner-Wright 1997; Conant and Collins 1998; Oldfield and Moriarity 1994; Wisconsin Herp Atlas Project 2007). Any further sampling for this species probably should be concentrated in that portion of the park.

We conducted targeted searches for a limited number of snakes and lizards in the MISS (Table 8). Of the species we targeted but did not observe, the five-lined skink (*Eumeces fasciatus*) and the prairie racerunner (*Cnemidophorus sexlineatus viridis*) could exist in the MISS, but probably only in limited locations based upon habitat availability, especially for the prairie racerunner. Of our targeted turtle species, we did not observe the midland softshell turtle (Table 8). Habitat

existed for this species in the MISS and further surveys would be necessary to be more confident in whether or not it is present. This, in fact, is true for practically all the unconfirmed amphibian and reptile species in the MISS, as our effort to confirm the presence of species we targeted was resource-limited and we did not target all unconfirmed species.

Conclusions

Not only are amphibians and reptiles important ecologically, but global amphibian and reptile declines are due to environmental changes associated with global change. Because of their sensitivity to such change, these taxa, and amphibians in particular, have potential value as indicators of ecological effects that could be affecting other organisms as well. Based upon our results, most all the amphibian species we expected to find were present, as were many of the turtles. However, we do not know if these populations are increasing or decreasing in distribution and abundance or if exposure to pesticides and emergent diseases at some locations, among other factors, is impacting them. We have provided baseline information here that we hope will enable NPS and FWS biologists to better understand these issues over the long term through further research. At the least, we hope the NPS Inventory and Monitoring Program in the GLKN will build upon these efforts by continuing the amphibian monitoring they have begun in select parks and the FWS will do the same with their ongoing efforts in the UMR.

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Appendix A. Table of contents for the data supplement.

The tables and figures listed below are provided in a Data Supplement to this report, which is available for download as a separate document. These tables and figures provide further details and data pertaining to the work described in this report.

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Table 22. Occupancy analysis results by species from all ARMI management units including the Neal Smith National Wildlife Refuge from 2004-2005 and the Upper Mississippi National Fish and Wildlife Refuge, the St. Croix National Scenic Riverway and Voyageurs National Park from 2002-2005.

Table 23. Number of metamorphs per species caught during metamorph abnormality surveys for the Neal Smith National Wildlife Refuge (NS) from 2004-2005 and the Upper Mississippi National Wildlife and Fish Refuge (UMR), the St. Croix National Scenic Riverway (SACN), and Voyageurs National Park (VOYA) from 2003-2005.

Table 24. General health screening and abnormality specimens sent to USGS's National Wildlife Health Center from the Upper Mississippi National Wildlife and Fish Refuge from 2002-2005.

Table 25. General health screening and abnormality specimens sent to USGS's National Wildlife Health Center from the St. Croix National Scenic Riverway from 2002-2005.

Table 26. General health screening and abnormality specimens sent to USGS's National Wildlife Health Center from Voyageurs National Park from 2002-2005.

Table 27. Batrachochytrium dendrobatidis detection results for pooled swabs of metamorphs from sites within the Upper Mississippi National Wildlife and Fish Refuge and submitted to Pisces Molecular Lab for PCR tests during 2006 and 2007.

Table 28. Batrachochytrium dendrobatidis detection results for pooled swabs of metamorphs from sites within the St. Croix National Scenic Riverway and submitted to Pisces Molecular Lab for PCR tests during 2006 and 2007.

Table 29. Batrachochytrium dendrobatidis detection results for pooled swabs of metamorphs from sites within Voyageurs National Park and submitted to Pisces Molecular Lab for PCR tests during 2006 and 2007.

Table 30. Batrachochytrium dendrobatidis detection results for pooled swabs of metamorphs from sites within Wisconsin Department of Natural Resources Blanchard's cricket frog sites and submitted to Pisces Molecular Lab for PCR tests during 2006.

Table 31. Turtle species observed within the St. Croix National Scenic Riverway during daytime surveys, basking turtle surveys, and targeted searches from 2002-2005.

Table 32. Turtle species observed within Voyageurs National Park during daytime surveys, basking turtle surveys, and targeted searches from 2002-2005.

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